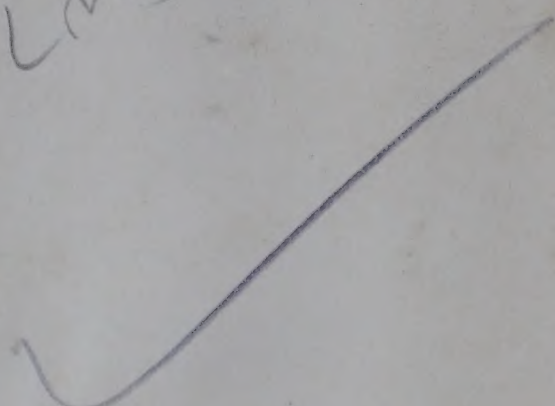# PROCEEDINGS *of* THE SECOND CONFERENCE ON RESEARCH
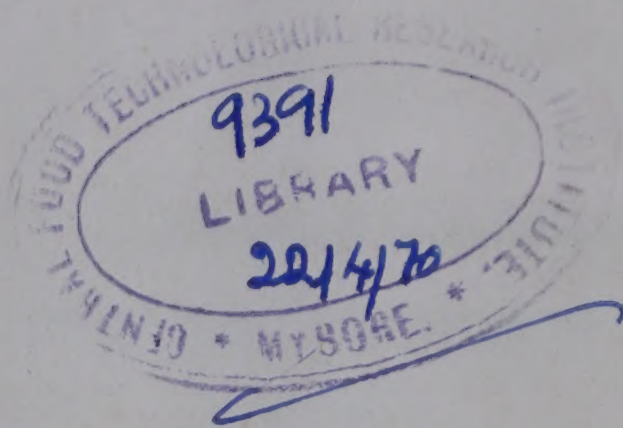
Conducted under Sponsorship of

THE COUNCIL ON RESEARCH

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AMERICAN MEAT INSTITUTE

at

THE UNIVERSITY OF CHICAGO
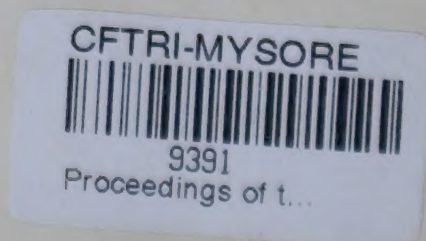
MARCH 23 AND 24, 1950

CHICAGO, ILLINOIS

Table of Contents

I

VITAMIN B_{12}

C. A. Elvehjem
Department of Biochemistry
University of Wisconsin

Any consideration of the biological significance of vitamins brings to mind two very interesting facts. One is the almost universal role of certain vitamins in living matter and the second is the striking difference in the requirements of two very closely related types of living cells for a given vitamin. Although interest in vitamins first developed in the field of animal nutrition, the work had not progressed very far before it was realized that the growth factors for yeast, that had been described at the beginning of the century, were closely related to vitamins. Although yeast and plant products were used as starting materials for the isolation of vitamins, it took some time to accept the importance of the role of vitamins in the metabolism of higher plants. We need only go back to 1938 in the book on Vitamin B_1 by Williams and Spies to find the following statement: "Since it (thiamine) is indispensable for higher animals, for insects, and for higher plants, it surpasses all other specific substances in the scope of its biological significance."

Today many of the vitamins can be placed in the same category. On the other hand it is also possible to describe certain vitamins which do not carry over from one form of living matter to another. Carotene, which is so universal in higher plants, cannot be found in most microorganisms or animal products. Neither carotene nor vitamin A is found in insects. Vitamin D is absent from plant material but can be produced through irradiation of the fried material. Vitamin C which is so abundant in plants and definitely present in most animal tissues, cannot be found in the lower organisms.

One of the best examples is the complete absence of vitamin B_{12} in higher plants and yeast and the very significant amount present in animal products, certain molds, and bacteria. As far as I know this is the only example of a nutrient which cannot be obtained from the usual plant foods. Vitamin D may be placed in a similar group but a deficiency of this vitamin can at least be compensated for through sunlight. I know of no place where the importance of the animal can be emphasized to a greater extent than in the supply of vitamin B_{12}. Hence, the term "animal protein factor" or perhaps even better "animal factor" is a true designation of this vitamin. For 25 years or more the packing plants and pharmaceutical houses have done an excellent job in supplying this life saving nutrient for thousands of individuals suffering from pernicious anemia.

Since the animals which supply the livers for making liver extracts live on plant material, what is the source of vitamin B_{12} for the animal? In those areas of the world where the human population lives to a large

1

extent on plant products, what is their source of vitamin B_{12}? I shall try
to answer some of these questions and discuss in a general way the sig-
nificance of vitamin B_{12} in nutrition.

What is vitamin B_{12}? Rickes and coworkers (1) defined vitamin B_{12}
as a compound isolated in crystalline form from liver which is highly ef-
fective in the treatment of pernicious anemia and also active for the grow
of Lactobacillus lactis. I realize that there may be more than one form of
vitamin B_{12} but for our discussion today I think it is unnecessary to go in
these details. There may also be arguments regarding the specificity of
vitamin B_{12} in the treatment of pernicious anemia but here again I think
we are generally agreed that it is the true antipernicious anemia factor.
I would, however, like to point out that folic acid may be equally as im-
portant as vitamin B_{12} in the handling of certain forms of pernicious ane-
mia.

In order to understand the mechanism of action or significance of vit:
min B_{12} we must know something about its distribution in nature. In orde:
to determine or study this distribution we need accurate and reliable assa
procedures. I have already mentioned that the Merck workers used Lacto
bacillus lactis as the assay organism. In our work we have used Lactobac
illus leichmannii for the estimation of vitamin B_{12}. Very briefly, we used
the basal medium of Skeggs et al. (2) with certain modifications. These
modifications include mainly the addition of cysteine and fumarate to the
basal medium. Using this procedure we have obtained satisfactory stan-
dard curves, low blanks, and satisfactory recovery values (3). In certain
cases we found it necessary to treat the samples in order to release all
of the vitamin B_{12} activity. In order to do this a 24-30 hour digestion of
the homogenized sample was treated with trypsin and the digestion car-
ried out at the neutral point. Autolysis did not give full release of activ-
ity or else destruction occurred. Pancreatin was found to give satisfac-
tory release but its used resulted in extremely high blanks.

In order to check the accuracy of our microbiological assay we have
measured the vitamin B_{12} activity in a number of biological materials
through the use of both the microbiological method and the rat assay. The
ration used in the rat assay was the corn-soybean ration fortified with all
the known vitamins except vitamin B_{12}. The ration contained .06% iodin-
ated casein, and on this ration weanling rats show a growth plateau after
2 weeks. After the two week preliminary period the materials to be tested
are added and the growth response during the following two weeks is mea-
sured. A standard curve is obtained by feeding given levels of vitamin B_{12}
and referring the growth of the rats on the assay samples to this standard
curve. The amount of vitamin B_{12} present can be calculated.

In Table I a few figures are given which show the results obtained
with the microbiological assay and the rat assay. You will observe that
in most cases there is excellent correlation between the two procedures.
Before leaving the rat assay method I want to emphasize that there may
be something in the corn-soybean ration which is not present in synthetic
rations. In other words, when we use a synthetic ration plus iodinated
casein the response to vitamin B_{12} is not nearly as great as that obtained
on the corn-soybean ration (Table II). We, therefore, agree with the re-
cent observations of Ershoff (4) and of Betheil and Lardy (5) that natural
materials such as liver contain a factor in addition to vitamin B_{12} which
is necessary to counteract the effect of iodinated casein. However, the
need for this additional factor does not interfere in the assay which we us

2

The vitamin B_{12} content of a number of biological materials is given in Table III. It is clear that beef liver and kidney are rich sources of vitamin B_{12}. Heart and adrenal tissue are also quite rich but spleen (hog) seems to be more variable. Chick gizzard is also a good source. The presence of this vitamin in these tissues must be dependent upon the synthetic ability of the organisms in the intestinal tract of the different animals. Greatest emphasis has been placed on the production of vitamin B_{12} in the rumen of ruminants. Actually the amount present in desiccated sheep rumen contents is greater than that in dried liver. It is as high as a dried defatted liver powder (VioBin). The amount in cow manure is not as great as in chicken manure or guinea pig feces Part of this may be due to the high fiber content of cow manure and partly to the fact that the vitamin synthesized in the rumen may be abosrbed and does not appear in the feces. So far we have found chicken manure to be one of the richest sources. Definite quantities can be observed in milk, casein, cheese and egg yolk. The amount in casein depends upon the method used for preparing the casein.

Cobalt is undoubtedly needed for the synthesis of vitamin B_{12} in the tract but under normal conditions the small amount needed is present in the ration consumed. We have added extra cobalt to the ration of rats but no increase in growth or vitamin B_{12} content of the liver could be observed. This test was made with the corn-soybean ration with added iodinated casein. Some effect may be observed for a synthetic ration low in cobalt.

If we accept a figure of 3γ of vitamin B_{12} as the requirement for maximum hematopoiesis in human pernicious anemia (6) then only six grams of dry liver would be needed. However, the above figure holds only for parenteral administration. In the presence of gastric juice 5 of vitamin B_{12} administered orally has given a good response. In other words, 10 gm. of dry liver should give maximum response in the presence of gastric juice. In the absence of gastric juice 25 to 35γ of vitamin B_{12} weekly has failed to produce a hematopoietic response in patients having pernicious anemia in relapse (7). This would be equivalent to 50 to 70 gm. dry liver or 150 to 200 gm. fresh liver per week. Since this amount of fresh liver is often effective, liver may contain something which improves utilization of the vitamin B_{12}. It is also well known that the hematopoietic activity of liver as well as crude extracts (8) can be greatly increased when ingested with normal human gastric juice.

When we turn to muscle meat we find a fairly high and rather constant level of vitamin B_{12}. 5-10γ per 100 grams. If the vitamin B_{12} administered were utilized as well as by injection, a daily serving of beef would supply enough vitamin B_{12} to produce a significant response in pernicious anemia. However, since 35γ are inactive orally without gastric juice, it is not surprising that Castle and Townsend (9) found beef muscle ineffective without incubation. It seems clear that beef muscle contains sufficient vitamin B_{12} to account for the extrinsic factor activity of muscle meats. The action of the intrinsic factor in gastric juice may not be merely the release of vitamin B_{12} from the food or reaction with the extrinsic factor but also in improving the absorption of vitamin B_{12} from the tract whether the vitamin comes from the food or the bacterial cells. Berk et al. (10), as well as Bethell and associates (II), have found large fecal excretion of vitamin B_{12} in cases of untreated pernicious anemia. The conditions in the intestinal tract of these cases are abnormal. In normal humans there should be sufficient gastric juice to allow ade-

3

quate absorption of vitamin B_{12} and under these conditions meat and fish should supply ample amounts of the vitamin to meet the requirements. Al of our work with rats and chicks indicates that the same response is obtained when the pure vitamin or concentrates of the vitamin are given orally or parenterally. In these animals we are dealing with normal condition Castle and coworkers have also reported that a greater response is obtained by treating muscle containing 1γ of vitamin B_{12} with gastric juice than by treating 5γ of the crystalline vitamin.

Careful studies on the absorption and utilization of vitamin B_{12} are complicated by the fact that a number of factors affect the amount of vitamin B_{12} synthesized in the tract and by the fact that factors in addition to the known vitamins may still be involved. Soon after our microbiological assay for vitamin B_{12} was developed we found that folic acid had a profou effect upon the storage of vitamin B_{12} in the liver of chicks. Figures in Table IV show the amount of vitamin B_{12} and folic acid in the livers of chicks receiving our regular synthetic ration supplemented with different levels of folic acid. The addition of 50γ of folic acid per 100 gm. of ration produced a three-fold increase in the vitamin B_{12} content of the liver but only a two-fold increase in the folic acid content. With 200γ of folic acide per 100 gm. of ration there was no further increase in the amount of vitamin B_{12} but this addition produced a greater growth response and a larger concentration of folic acid in the liver. It is difficult to determine whether the effect of folic acid is on the synthesis of vitamin B_{12} in the tract, in the animal body, or upon the utilization of vitamin B_{12}. Additional figures are given in Table V. It is evident that the addition of 0.2γ of vitamin B_{12} per chick per day did not produce as much of this vitamin in the liver as the addition of 200γ of folic acid per 100 gm. of ration. The addition of vitamin B_{12} alone increased the folic acid content of the liver. When vitamin B_{12} was given along with vitamin C a significant increase in both vitamin B_{12} and folic acid occurred. In all of our work very little additional growth response has been obtained when vitamin B_{12}, either in the pure form or as liver concentrate, is added on top of 200-500γ of folic acid per 100 gm. of ration. Folic acid may stimulate sufficient synthesis of vitamin B_{12} or the requirement of vitamin B_{12} may be decreased sufficiently in the presence of folic acid to reduce the amount needed in the ration. If folic acid is needed for the synthesis of thymine and vitamin B_{12} is needed for the synthesis of thymidine it is possible that in the presence of excess thymine less vitamin B_{12} may be needed for the production of thymidine. However, we have been unable to observe any effect of thymine in our rat assays using the iodinated corn-soybean ration. However, the production of thymidine within the tissues may have a different effect than the oral administration of this compound. Since some beneficial effect has been reported, especially in swine, through the use of alfalfa leaf meal, one wonders why this effect is observed, since alfalfa is apparently either devoid or very low in vitamin B_{12}. The observed effect may be due to an indirect stimulation due to the folic acid supplied by the alfalfa. We know relatively little about where B_{12} is produced in the tract and what organisms are involved in this synthesis.

In Table VI a few results are presented which indicate that the administration of a sulfa drug may reduce the synthesis of folic acid in the ceca of chicks almost completely, without affecting the production of vita-

4

min B_{12}. Again we do not know whether different organisms are affected or whether sulfa drugs prevent the synthesis of folic acid in certain organisms, without affecting the production of vitamin B_{12}.

Many years ago we observed that rats grew remarkably well on whole milk diets fortified with iron, copper, and manganese. We have been interested in following these studies to see if the addition of folic acid and vitamin B_{12} would improve the growth of animals on milk diets. A few typical results obtained with goat's milk and cow's milk are given in Table VII. With goat's milk we have observed an improved growth with folic acid alone and vitamin B_{12} alone and a better growth when both vitamins are added. In the case of cow's milk no significant effect was produced by adding these vitamins. An analysis of the liver from these animals also gives interesting results since the addition of folic acid to cow's milk increases the B_{12} content of the liver but this increase is not observed with goat's milk. Part of this may be due to the fact that goat's milk is lower in vitamin B_{12} than cow's milk. Furthermore, the addition of folic acid to cow's milk produces a greater increase in the folic acid content of the liver than when goat's milk is used. In both cases there is an increase in folic acid when this vitamin is supplemented with vitamin B_{12}. These results are presented to show that the diet may have a significant effect on the absorption and storage of vitamin B_{12} even though no changes in growth response can be observed.

For several years we have observed that rats fed on our regular synthetic diet or on a purified ration, in which beef supplies the protein, excellent reproduction is observed. When pork is used as the source of protein the reproduction record is more erratic. We thought that vitamin B_{12} might explain this difference. However, upon repeating our work with and without added vitamin B_{12} we find no difference in reproduction. However, in Table VIII we find that these different rations have quite a marked effect on the concentration of vitamin B_{12} in the livers and feces of the rats. These determinations were made on female rats shortly after parturition and therefore the figures in all cases are lower than those observed in young growing rats. There may be some possibility that choline enters into this effect since Schaefer et al. (13) have shown that choline may have some sparing effect on vitamin B_{12}. However, the casein ration contained 1% of choline.

I may also mention that studies with growing mink indicate that other factors are involved in addition ot vitamin B_{12}. Until all these factors are identified it is difficult to understand the exact mechanism of action of vitamin B_{12}. However, in conclusion I believe it is evident that animal products are rich sources of vitamin B_{12}. Organ tissues are especially rich with somewhat smaller amounts in muscle meats, milk, and eggs. The source of the vitamin must be largely dependent upon intestinal synthesis of vitamin B_{12}. The synthesis and absorption of the vitamins are still not thoroughly understood. Further studies on different diets may eventually help to explain why certain individuals develop a deficienty of vitamin B_{12} even though their diet is not devoid of this vitamin. Certainly we have no indication that the use of meat or animal products as a source of vitamin B_{12} has anything but a beneficial effect in the maintenance of optimum health.

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Table I -- The Rat Growth Assay Compared with the Microbiological As-
say for Determining Vitamin B_{12} Activity

Material	Vitamin B_{12} Content, γ/100 gms. dry weight	
	Rat assay	Microbiological assay
Beef round (cooked)	5.5	5.0
Beef tongue (cooked)	5.5	7.6
Pork shoulder (cooked)	0.9	0.7
Pork ham (cooked)	2.2	2.9
Veal (cooked)	3.6	3.0
Horsemeat (cooked)	7.5	7.0
Fish solubles	40.0	25.0
Beef liver	50.0	47.0
Tomato juice	0.0	0.0

Table II -- Effect of Basal Ration on Response of Rats to Vitamin B_{12}

Ration	Av. weekly gain 4 week period
	gms.
Casein-sucrose basal (0.3% iodinated casein)	16
Casein-sucrose basal (0.3% iodinated casein) + 0.2γ B_{12}/day	20
Corn-soybean basal (0.3% iodinated casein)	16
Corn-soybean basal (0.3% iodinated casein) + 0.2γ B_{12}/day	36
Casein-sucrose basal (0.3% iodinated casein) + 45% corn meal + 0.2γ B_{12}/day	30

Table III -- The Vitamin B_{12} Content of Biological Materials

Materials	γ/100 gms. dry weight
Beef liver	50.0
Beef kidney	50.0
Beef heart	25.0
Chicken gizzard	30.0
Hog spleen I	0
Hog spleen II	22.0
Hog spleen III	9.0
Hog adrenal	15.0
VioBin liver powder	100.0
Crude commercial casein	3.0
Casein prepared from skim milk	7.0
Cow's milk (dry)	2.0
Cheddar cheese	2.5
Egg Yolk	3.0
Fish solubles	15.0
Desiccated sheep rumen content	100.0
Guinea pig feces (synthetic ration)	230.0
Chicken manure	450.0
Goat manure	20.0
Cow manure	47.0
Beef round (fresh)	7.9
Pork shoulder (fresh)	6.5
Mutton (fresh)	8.8
Chicken breast (fresh)	5.3
Chicken leg (fresh)	5.2
Salmon	8.5
Oysters	15.0
Beef round (cooked)	5.0
Beef tongue (cooked)	7.6
Pork shoulder (cooked)	3.0
Pork hocks (cooked)	0.1
Pork ham (cooked)	3.0

Table IV — Effect of Level of Folic Acid on Chick Growth and the Folic Acid and Vitamin B_{12} Content of the Livers

Supplement	Gm. wt. gain	γB_{12}/ liver	γFolic acid/liver
None	19	0.05	2.82
50γ Folic acid/100 gm. ration	73	0.16	4.03
200γ Folic acid/100 gm. ration	126	0.15	15.60
500γ Folic acid/100 gm. ration	152	0.08	14.05

Table V — Effect of Vitamin B_{12}, Folic Acid and Vitamin C on Chick Growth and the Folic Acid and Vitamin B_{12} Content of the Livers

Group	Supplement	Gm. wt. gain	γB_{12}/ liver	γFolic acid/liver
Basal	None	19	0.05	2.82
	Vitamin B_{12} (0.2γ/day)	40	0.12	5.70
	Vitamin C (100 mg.%)	33	0.06	4.65
	Vitamin B_{12} (0.2γ/day) + Vitamin C (100 mg.%)	59	0.24	10.04
200γ Folic/acid 100 gm. ration	None	126	0.15	15.60
	Vitamin B_{12} (0.2γ/day)	131	0.15	17.66

Table VI — Effect of Sulfasuxidine on Vitamin B_{12} and Folic Acid Content of Ceca from Chicks

Group	Supplement	Folic acid γ/gm. dry wt.	Vitamin B_{12} γ/gm. dry wt.
50γ Folic acid/100 gm. of ration	None	2.65	4.50
	Vitamin B_{12} (0.1γ/day)	9.57	6.25
	Vitamin C (100 mg. %)	4.75	3.58
50γ Folic acid/100 gm. ration + sulfasuxidine (1%)	None	0.00	4.30
	Vitamin B_{12} (0.1γ/day)	0.00	5.10
	Vitamin C (100 mg. %)	0.88	3.42

Table VII — The Effect of Folic Acid and Vitamin B_{12} on Growth and
Liver Vitamin Content of Rats Fed Mineralized Milk

Milk	Daily additions to mineralized milk per rat	Average weight gain in 5 weeks gm.	Average liver vitamin level (γ/100 gm. dry liver)	
			Folic acid	Vitamin B_{12}
	None	126	186	3.0
Goat's	+ Folic acid (50γ)	142	546	3.0
milk	+ Vitamin B_{12} (0.1γ)	154	240	9.0
	+ Folic acid (50γ) + Vitamin B_{12} (0.1γ)	176	912	9.0
	None	169	192	15.0
	+ Folic acid (50γ)	176	1116	15.0
Cow's	+ Vitamin B_{12} (0.1γ)	170	384	21.0
milk	+ Folic acid (50γ) + Vitamin B_{12} (0.1γ)	181	2430	33.0

Table VIII -- The Vitamin B_{12} Content of the Liver and Feces of Albino
Rats after Parturition

Ration	Vitamin B_{12} concentration (γ/100 gm. dry weight)	
	Liver	Feces
Beef	8.4	114.0
Beef plus 0.1γ vitamin B_{12} daily	16.5	191.0
Pork	6.9	51.5
Pork plus 0.1γ vitamin B_{12} daily	9.0	65.5
Casein	1.5	32.8
Casein plus 0.1γ vitamin B_{12} daily	2.0	35.8

II

MEAT THROUGHOUT LIFE

A. J. Carlson
Department of Physiology
The University of Chicago

I was led to undertake this line of work by the investigations of Dr. McKay on rats at Cornell where he seemed to show that contrary to the evidence we have with man, undernutrition lengthened the life of rats. I know McKay---I know his work---I saw his rats.

Did undernutrition lengthen the life span or did his control group by eating in excess shorten the life span? We tried to settle that in various ways by restriction of foods, partial fasting, so that there was no impairment of growth. Under those conditions, the animals restricted in that way lived longer than the control group on the same diet eating ad libitum. Those rats apparently behaved very much like human beings who don't have to struggle for their food---they eat and sit.

Now, the day the rats didn't have anything to eat they ran. We hadn't figured on that. We should have, but Dr Correll, I don't think was right when he said that any real investigator knows the answer before he starts out with the experiment. If that is the definition, I am not a real investigator.

We finally settled on to the principle of adding indigestible roughage to the food to the point where the good diet ad lib. couldn't be overeaten. They simply didn't have big enough stomachs. Under those conditions they eat and sit, but they definitely live longer in better condition than the rats who eat the same diet concentrated without roughage.

In this work, we decided to make a test to repeat some of the work done by Slonaker at Stanford some forty years ago, and some later work, particularly by Chinese investigators in China on the effect on the life span of essentially an omniverous mainly meat diet and the best selected, I mean the best so far as we know now, wholly vegetarian diet on the rats. There is some discrepancy between these results and those of Slonaker on the nearly vegetarian diet that impaired growth and very definitely shortened the life span. The Chinese, on the other had, got retardation of growth, but no shortening of the life span.

Well, we set out aided by funds from Swift & Company and did three studies (maybe they were not so wise) where we studied the growth, life span, fecundity and capacity to raise the young. The omnivorous, or mainly meat diet was 61 per cent boned veal; that is, dessicated calf, plus 3 per cent bone meal, and thirty one per cent corn starch, salt of course, and some cod liver oil.

In our first experiment, the vegetarian diet was ground up. I mean the grains were ground and the nuts; and these were stone ground whole wheat fifty per cent, peanut flour 10 per cent, wheat gluten flour, lima

11

bean flour, linseed meal, gluten meal, alfalfa meal, 5 percent brewers
yeast, salt, and supplemented with lettuce. Now, this was all ground up.

Maybe the mistake we made was not the ration, but the cod liver oil.
In the second series, we didn't grind anything, but let the rats select
what they wanted ad lib. of the following substances: corn — whole kernel,
wheat — whole kernel, peeled barley, rolled oats, sunflower seed, peanuts,
green beans, soybeans, defatted corn germ meal, defatted wheat germ
meal, brewers yeast, alfalfa meal, and of course table salt.

We thought this the best representative, probably the most nutritive
element of the vegetarian diet. We could not control or measure the food
consumption in this experiment very well. That's one of the things that
our experiment lacked. Now, what were the results over three years of
the life studies?

The animals on a wholly vegetarian diet, as compared to the omni-
vorous or largely meat diet, grew slower. As measured by tibia length,
the ultimate size was smaller, as measure by tibia length rather than
weight, which is partly a matter of adiposity. This, despite the fact that
on this omnivorous diet eating ad lib. the rats on that diet as compared
with the control had a shortened life span unquestionably as determined
by earlier work.

Secondly, the rats on the vegetarian diets, both of them, both times,
had fewer young, had reduced fertility, reduced capacity to raise their
young and continued to eat the offspring. That effect was increased, and
there was a very definite appearance of inability of the bones to grow.
In other words, rickets developed. In brief, the essentially meat diet
proved definitely superior to the best type of purely vegetarian diet we
could devise.

That the meat proteins are more complete in amino acid composition
than those of vegetable protein is pretty well established. But how do her-
bivorous animals in nature carry on without animal protein, or do they?
The rumen of the ruminants does something, to be sure. But there are
herbivors without a rumen. Is it possible that in nature wild animals and
primitive man get enough bugs, flies, roaches, to supply the minimal
need of animal proteins? But don't laugh. How many of you have seen
monkeys pick off the lice of their neighbor and eat them? I have. Now
that source of animal protein, of course, is eliminated by sanitation,
cooking and preparation, in modern human society. I'm sorry I took so
much time. I think there are criticisms that could be levelled against
our type of experiments. I should like to have them. Thank you.

III

DIRECT AND INDIRECT DETERMINATION OF THE NUTRITIVE VALUE OF PROTEINS

D. V. Frost
Abbott Laboratories
North Chicago, Ill.

In recent years, the development of new methods based on many novel principles (e.g. isotope dilution, microbiological assay, and partition chromatography) has revolutionized amino acid analysis. The methods are all micro in scale and experienced operators can obtain very good approximations of the amino acid content of sample proteins in just a few days. About 7 years ago, such a protein analysis would have required painstaking and patient effort for many weeks and the results would not have been as reliable as those by present methods.

Much has already been done with the new methods and the literature is replete with analyses on the essential amino acid composition of most important food proteins. Although there is not precise agreement on many of these analyses, one can generally judge which amino acids are present in seriously limiting amount from the nutritive standpoint. The arm-chair evaluation of the feeding value of proteins, based on the essential amino acid content, is bound to be an intriguing one, and it is the purpose of this paper to briefly explore some of the possibilites and limitations of such evaluations.

Just a word of history — it was the acute imbalance of certain proteins which actually led to the discovery of certain of the amino acids, and automatically led to establishment of their essential nature. Hopkins in 1906 corrected one of the primary deficiencies of the protein, zein, from corn by adding his newly discovered amino acid, tryptophan. This was an early important step in the then baby science of nutrition. In 1914 Osborne and Mendel immensely broadened the concept of the importance of individual amino acids. They showed that the addition of both lysine and tryptophan to zein very markedly improved the nutritive character of this protein. They also established that lysine is the primary deficiency of gliadin, the principal protein of wheat. The deficiency of lysine could also be corrected effectively by supplementing gliadin with proteins, such as meat proteins, which are high in lysine. Thus, the concept of limiting deficiencies and of supplementary effects between specific proteins is old. What is new is the great mass of information we now have on protein composition, specific amino acid requirements, and improved methods of animal assay.

Method of Mitchell and Block:

Many of you are acquainted with the studies of Mitchell and Block (1), in which whole egg was taken as a standard of nutritive excellence among proteins. The amino acid make-up of a large number of proteins was com-

13

pared with the amino acid composition of whole egg, which was arbitrarily assigned a value of 100%. The way in which the essential amino acid content of a given protein deviated from that of whole egg provided the basis for judging nutritional value. The greatest single amino acid deviation below the levels provided by whole egg was taken as the limiting deficiency, and a formula was devised to calculate the effect of the deficiency on the nutritive value of the protein. Comparison was then made of the estimated values with values determined by bioassay. The estimated and determined values agreed well in most instances and the study pointed up the possibilities of such estimations.

Standards Based on Patterns of Amino Acid Requirements:

Another obvious approach to estimation of the ability of proteins to satisfy essential amino acid requirements is to balance the composition of a protein against the established minimum requirements for growth, maintenance, or repletion. The minimum requirements for growth of rats has been carefully determined over a period of years by Rose (2). Very recently also, Rose (2a) has reported the requirements for maintenance of nitrogen balance in young adult men. Furthermore, Cannon and his collaborators (3) (3a), at the University of Chicago, have reported the minimum needs of the adult protein-depleted rat for a good rate of repletion. Following the practice established by Cannon, it is instructive to portray the ratios of these requirements by assigning an arbitrary value of 1 to trytophan. The comparisons are shown in Table 1.

As will be seen, the ratios vary one from the other to some extent, but are nevertheless all within a fairly limited range. One would perhaps expect that the ratios of requirements for maintenance would differ markedly from those for growth or repletion. The gratifying thing for the purpose at hand is that the ratios actually compare as closely as they do.

A very good rationale for the constancy throughout the animal kingdom of a specific pattern of amino acid requirements can be built up from the war-time study of Beach, Munks, and Robinson (5). They presented extensive data that tissue composition varies remarkably little between species. The pattern of amino acid composition of the muscle of frogs legs was shown to be essentially the same as that of chicken, codfish, or beef. Similarity in composition between milk and meat proteins was also indicated. The authors concluded that the composition of mammalian tissue itself provides a good pattern for dietary purposes.

Limitations:

Although the case for chemical evaluation of proteins appears good on a purely theoretical basis, there are very definite limitations. First of these is the factor of digestibility and assimilation of various proteins. This is known to vary considerably. The prime example is the protein of soybean, the methionine of which becomes more available with a certain degree of heat treatment. Although soybean is markedly improved by properly controlled heating, there is deterioration of value with more drastic heating. These effects are, however, not revealed by losses in amino acid composition. Likewise it has been shown that certain baking and drying procedures may alter the nutritive value of cereal products markedly. These heat changes in proteins are not reflected by the ordinary amino acid analyses. Thus one could easily be misled by consideration of the composition values alone.

It has been suggested that, to be fully utilized, all amino acids must

14

be liberated in the digestive tract at a rate permitting mutual supplementation (6). Recent experiments (7) have shown that the in vitro digestion of proteins by pancreatin provides a useful index to the in vivo availability.

A further obvious limitation pertains to the accuracy of the amino acid analyses themselves. This was encountered rather forcefully in our own laboratory when we found that the values of our analysis of whole egg protein differed significantly from the values reported by Mitchell and Block. There is generally a good deal of uncertainty remaining as to the precise accuracy of the various analytical methods. Standards have not been set and check methods are not themselves convenient or necessarily reliable. Thus, for various reasons, the precise formulation of protein value on the basis of amino acid composition appears to lack solid footing, and only approximations and indications appear warranted.

Some evidence has been presented suggesting the dietary importance of certain peptide components of proteins. I believe that at present the preponderance of evidence is that, for the higher animals, the free amino acids fulfill the needs for growth, maintenace, and repletion.

Also one may now completely dispel the notion that one would need to consider the supply of certain non-essential amino acids. Work from at least three laboratories has now shown that rats do very well with only the 10 essential amino acids plus a source of ammonia nitrogen (8) (9) (10). In our laboratory with adult depleted rats, it was possible to get optimum results with only 9 essentials and a source of ammonia, dispensing entirely with arginine.

It should be noted that rats have a higher proportionate requirement for the sulfur containing amino acids, presumably for hair growth, than do humans (13) (14). This fact must be considered in the interpretation of rat assay results. Mention need also be made of the supplementary relationship between cystine and methionine and between tyrosine and phenylalanine. The total amounts of these combinations in a protein must be considered to give the over-all adequacy with regard to methionine and phenylalanine.

Rat-Repletion Method:

The rat-repletion method of assay of protein value was developed by Cannon and his students (3), at the University of Chicago, as a tool for studying pathologic changes resulting from protein deprivation and regeneration. It turned out to be a rapid, convenient, and precise method of comparing proteins and some of the emphasis naturally turned to the nutritional side. We adopted the method with certain modifications as a means for measuring the nutritive value of liquid protein hydrolysates (11). Application of the method is best shown in the following figures.

Figure 1 shows the graded response to various levels of controlled nitrogen feeding. The lowest level, 120 mg. N per rat day, corresponds to a level of only about 7 per cent protein in the diet.

Figure 2 shows the dramatic effect of leaving out one of the essential amino acids completely.

Figure 3 shows the effect of varying the level of trypotophan in the critical range. Our results agree with those of Cannon in placing the minimum level for good recovery at about 14-15 mg. tryptophan per rat day.

Figure 4 shows the record of a group of 5 rats on lactalbumin standard (11a) in a regular control assay. Here the animals were used through 8 cycles of assay. This procedure is in use in our laboratories for rou-

15

tine control of the nutritive value of an intravenous protein hydrolysate. Five day assays are run each week on the five production lots. The rats are used for repeated assay, which represents a saving in animals, in time, and in handling.

Rutgers Reference Proteins:

An excellent opportunity was provided to compare methods of protein evaluation by the setting up at Rutgers University of a series of reference proteins covering a wide range of protein value. These proteins were then made available to various groups for assay. Some of the results have already appeared (11a) (12) (13). The study also included collaborative microbiological amino acid assays of the same proteins.

The results of our own analysis with the Rutgers reference proteins are seen in Table 2. The values shown are in terms of the per cent of Cannon's minima supplied by each protein at a definite feeding level. One could predict from this which proteins would be expected to promote a good response and which should fail. The results are shown in Table 3, together with the limiting deficiency. As will be seen, the amino acid data served fairly well to predict the result.

Practical Application:

Knowing the essential amino acid composition of proteins makes possible their strategic combination to achieve good nutritive balance. There is also the possibility of supplementation with synthetic amino acids or concentrates. Thus far, only methionine, among the pure amino acids, has been produced at a cost sufficiently low to warrant commercial use on a large scale. Rations based on plant proteins, particularly soybean, peanut, and cottonseed, are markedly improved by judicious fortification with methionine.

Other grain proteins are more specifically deficient in lysine. Large scale fortification of wheat products with lysine has not yet achieved practical importance although it has been the subject of much study.

We have had occasion to study the nutritive value of whole blood, which is deficient both in methionine and in isoleucine. The two principal proteins of blood, globin and blood albumin, are very nearly devoid of isoleucine, but have a high content of lysine. Thus, the addition of whole blood to grain proteins is in the right direction to correct the lysine deficit. In turn, the isoleucine of the grain partially corrects the lack of this amino acid in blood. Both whole blood, and wheat and corn products are high in leucine. The mixed leucine by-products available on a large scale from glutamic acid manufacture are also possibilities for the nutritive improvement of whole blood. To be maximally useful, these concentrates should provide a preponderance of isoleucine rather than leucine. The possibilities here are intriguing from various standpoints because of the large supply and low price of whole blood as a protein source.

In summary, it is clear that new methods permit a fairly thorough and conclusive assessment of the nutritive value of a protein in a short period of time. Amino acid analysis is chiefly useful to determine the adequacy of each of the essential amino acids in the particular pattern presented by a specific protein. The animal assays are, however, more conclusive than the indirect estimation of nutritive value. There is little to chose between the two methods of approach on the basis of convenience and rapidity. In a thorough analysis, the two approaches are mutually complementary, providing a clear picture of the net nutritive value to-

gether with a good indication as to which amino acids are limiting and to what degree.

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Table I.

	Rat Growth Requirement (2)		Rat Repletion Requiement (3)		Requirement for N Balance in Adult Humans (3a)		Calculated Requirements for N Balance in Adult Humans (4)	
	Diet %	Ratio	Mg./Day	Ratio	Gms./Day	Ratio	Gms./Day	Ratio
Tryptophan	.2	1	14.5	1	.25	1	0.4	1
Phenylalanine	.7	3.5	45	3.1	1.1	4.4	1.4	3.5
Leucine	.8	4	72.5	5	1.1	4.4	1.7	4.2
Isoleucine	.5	2.5	61	4.2	0.7	2.8	1.2	3.0
Methionine	.6	3.0	39	2.7	1.1	4.4	0.5	1.3
Threonine	.5	2.5	43	3.0	0.5	2.0	1.0	2.5
Lysine	1.0	5	58	4.0	0.8	3.2	0.8	2.0
Histidine	0.4	2	21	1.4	---	---	0.5	1.3
Valine	0.7	3.5	51	3.5	0.8	3.2	1.1	2.8

Table II -- Profile of Rutgers Reference Proteins as Compared with the Requirement for Each Amino Acid for Maximal Rate of Repletion (Cannon)*

Essential Amino Acid	Repletion Minima* per 150gm. rat/day mg.	Per Cent of Requirement Supplied by Each Protein — at Level of 120 mg. N per Rat Day					
		Egg Albumin %	Casein %	Defatted Whole Egg %	Peanut Flour %	Wheat Gluten %	Defatted Beef Muscle %
Tryptophan	14.5	97	69	97	77	69	83
Phenylalanine	45	111	96	98	71	82	64
Leucine	72.5	97	101	94	58	68	77
Isoleucine	61	70	70	70	43	52	47
Methionine	39	79	64	67	13	26	44
Threonine	43	84	79	88	42	44	79
Lysine	58	77	91	74	32	20	103
Histidine	21.5	93	116	75	73	71	126
Valine	51	114	108	116	53	55	71

*Values slightly revised from former published values (Cannon '48); communication from Dr. L. E. Frazier and Dr. P. R. Cannon, who kindly made the values available for this comparison.

The values underlined indicate the probable limiting amino acids and the severity of the limitations -- the limitations in methionine cannot be interpreted clearly because of the complemental effect of cystine, which is present in varying amounts in the different proteins.

Table III – Rat-Repletion Responses to Proteins at Two N Levels. Limiting Amino Acid Deficit Calculated from Essential Amino Acid Content.

| Protein Supplement | Repletion Response | | Limiting Amino Acid | % of Requirement Supplied* |
	at .12 gm. N/day gm.	at .24 gm. N/day gm.		%
Egg Albumin	38 ±3.5	80 ±4.9	Isoleucine	70
Whole Egg (defatted)	33 ±1.4	66 ±3.9	Isoleucine	70
			(Methionine	44
Beef Muscle (defatted)	31 ±1.4	46 ±3.5	(Isoleucine	47
			(Methionine	64
Casein	31 ±2.9	45 ±3.7	(Tryptophan	69
Peanut Flour	8 ±6.3	32 ±2.7	Methionine	13
Wheat Gluten	9 ±2.3	19 ±2.4	Lysine	20

*The milligram quantities of each of the essential amino acids supplied in an amount of protein containing .12 gm. N was calculated from the amino acid composition data for each protein. In each case, as shown, at least one of the essential amino acids was present at sub-optimal level as compared with Cannon's minima.
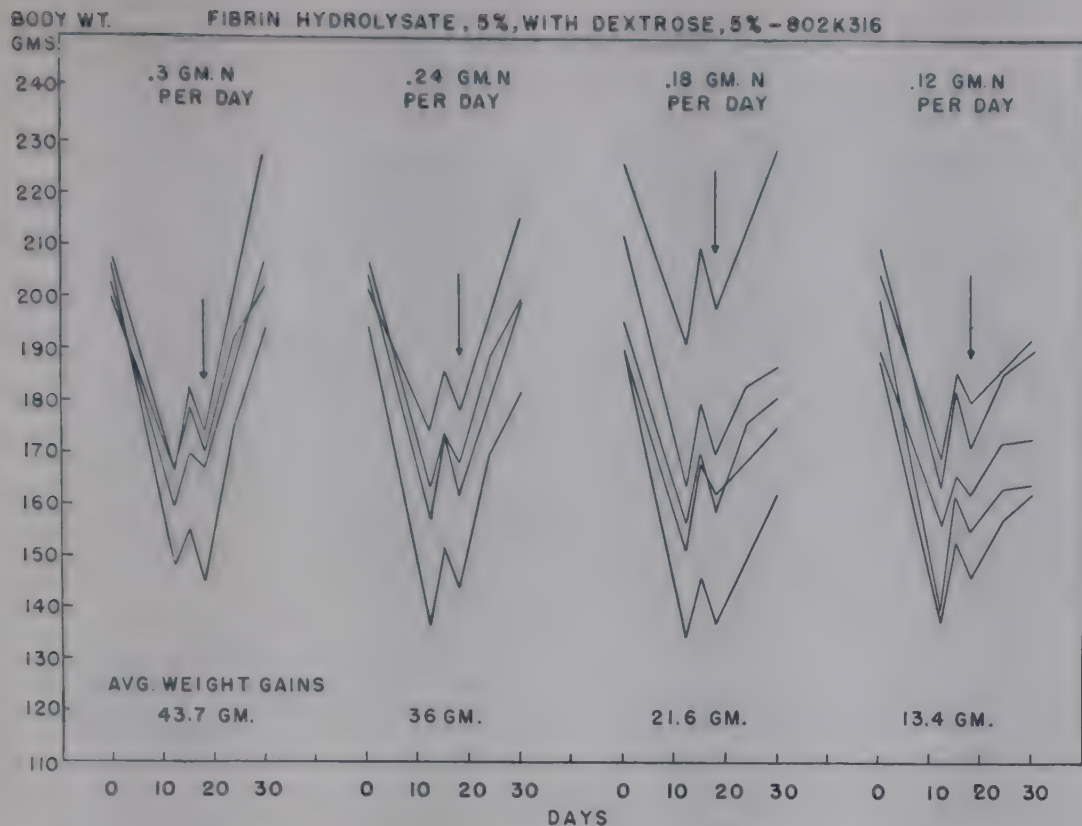
20

Figure 1. The arrows indicate the point at which feeding the fibrin hydrolysate at the four different intake levels was started. All rats were prepared for assay by a 12-day depletion period, a 3-day drinking trial on a standard fibrin hydrolysate, and a 3 day redepletion. From: J. Biol. Chem. 175, 635 (1948).
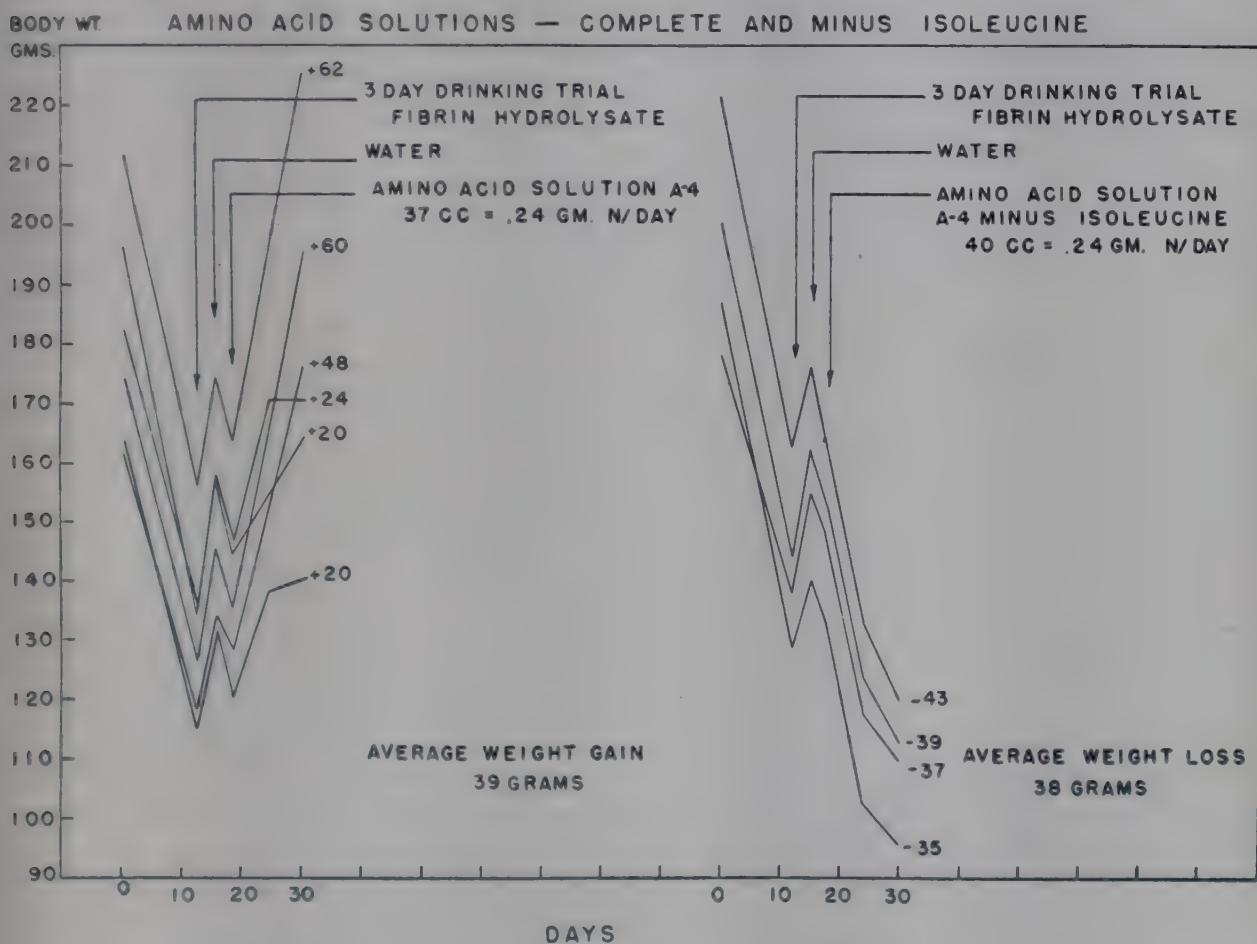

Figure 2. Weight changes of individual rats during 12-day depletion, 3-day orientation to liquid feeding, 3-day redepletion and final assay periods. Assay of a 5% amino acid solution patterned after the amino acid composition of casein (corrected for insolubility of tyrosine, cystine, and glutamic acid) versus the similar mixture minus isoleucine. From: Proc. Soc. Exp. Biol. & Med. 68, 51 (1948).
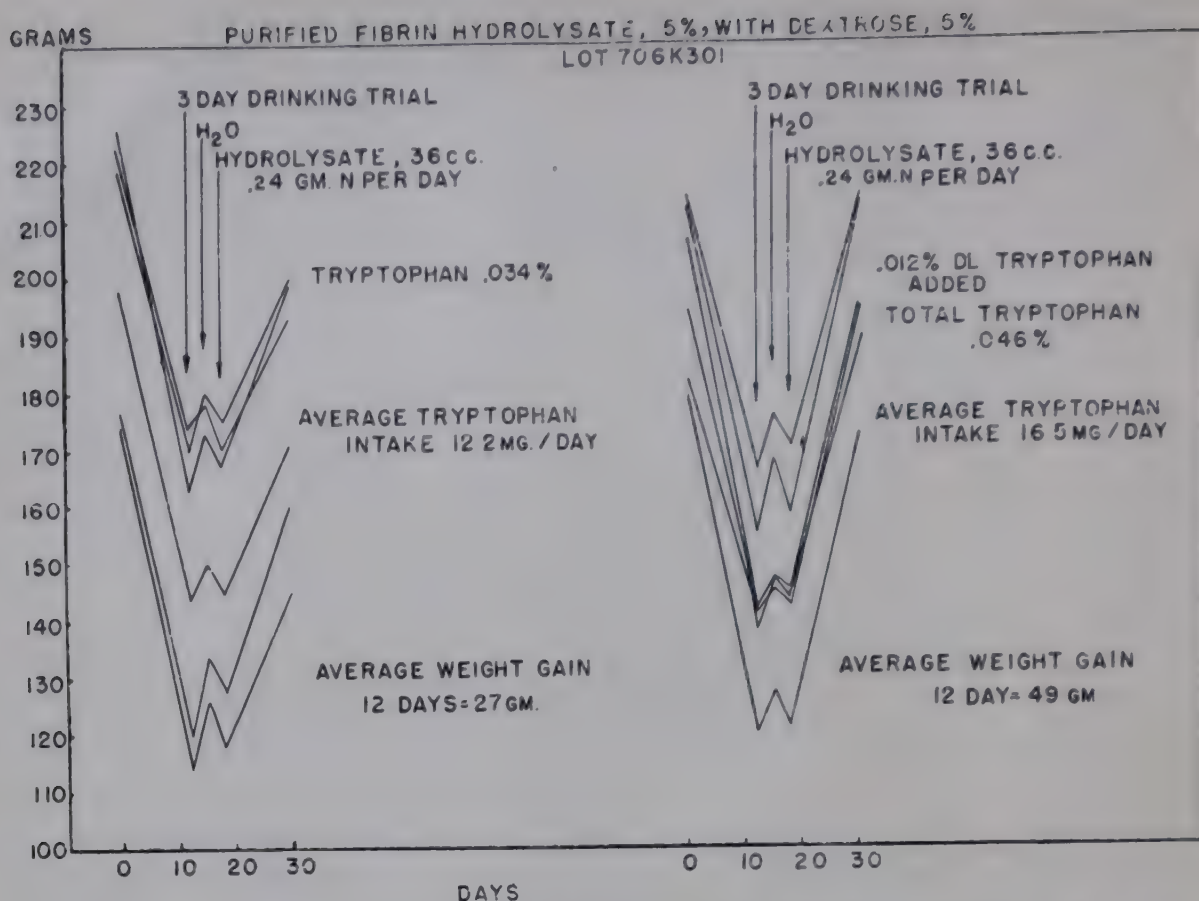
Figure 3. Weight response of protein-depleted rats (males) to controlled feeding of a partial acid hydrolysate of purified fibrin alone and with 0.24 per cent DL-tryptophan added on the hydrolysate solids. The solutions fed contained 0.24 gm. of N per day. The assay includes a 3-day orientation period during which the liquid hydrolysate was fed ad libitum, followed by a 3-day depletion before the assay. From: J. Biol. Chem. 175, 642 (1948).
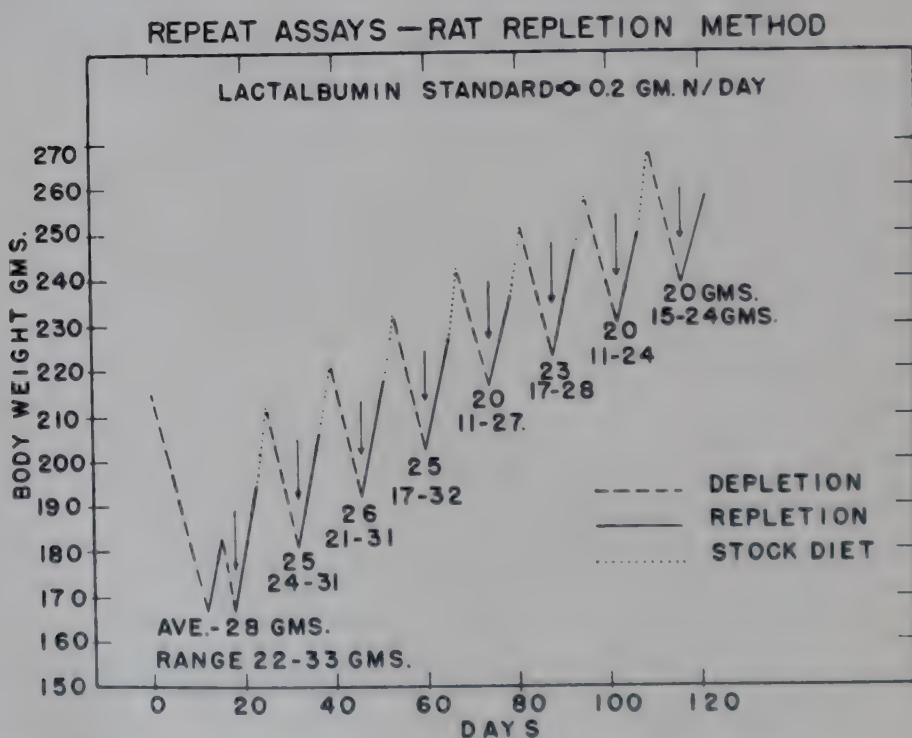
Figure 4. Average weight changes of 5 rats on lactalbumin as a standard in manufacturing control assay. First part of curve shows 12-day depletion on non-protein diet followed by three-day "drink trial" and three-day redepletion. Subsequent assay cycles consist of lactalbumin (5 days' repletion), stock diet (2 days) and redepletion (7 days). Averages and ranges of weight gain for each of the 8 assay periods are shown. From: J. Nutrition 39, 433 (1949).

22

IV

SOME NEW FACTORS IN ANIMAL FEEDING

W. M. Beeson
Department of Animal Husbandry
Purdue University

Introduction

Gentlemen, I have come here today to discuss with you rather brief-
ly a few new nutritional discoveries which I think may eventually affect
the practical feeding and production of farm livestock. I want you to know
that I consider it a privilege to speak before a group of individuals who
are so vitally interested in the production of livestock and the processing
of meat for human consumption. I think we must recognize at the outset
that people engaged in the field of animal research are rapidly revealing
new nutrients that are having a vital influence on the efficiency of live-
stock production and also on the quality of meat products that are offered
for human consumption.

Meat by-products have always played an important role in the nutri-
tion of farm animals and have helped tremendously in balancing the defi-
ciencies that are so apparent in corn.

Today I should like to discuss with you some of the research that
we are conducting at Purdue University to determine more specifically
the nutrients required for the growth and development of swine. This
discussion will be divided into three parts — the amino acid requirements
of swine, the "Animal Protein Factor" and the protein value of tankage.

Amino Acids

We have recognized grossly for many years the importance of high
quality proteins — or we might say balance of amino acids — as being
tremendously important in the growth and development of swine. We have
done a good job in using "shot gun" mixtures composed of animal and
plant protein supplements to balance the deficiency of amino acids con-
tained in corn. However, it has only been recently that we have discov-
ered the technique that is necessary to prove the accepted opinion that
quality of protein (balance of amino acids) is just as important, if not
more so, than quantity of protein for hogs. Crude protein analysis means
little to a hog because a single stomached animal apparently cannot syn-
thesize all of the amino acids which are essential for life. Rose, in his
research with amino acids at the University of Illinois, has rather con-
clusively shown that the amount of total protein required for growth and
development can be reduced drastically if the animal has a perfect dis-
tribution of the essential amino acids. Likewise, it is only logical to as-
sume that when the quantitative requirements of the essential amino acids
for swine are determined, the amounts of total protein required for
swine production should be materially reduced.

Recently at Purdue University we have shown that tryptophan, lysine and methionine are required for normal growth and development of weanling pigs. Pigs fed on semi- purified diets complete in all known factors, except tryptophan, lost 8 pounds in weight while litter mate pigs receiving 0.4% of DL-tryptophan gained 25 pounds in the 21-day period. These data conclusively shw that tryptophan is required for normal growth and development of the pig. Following this experiment a study was made to find out the quantitative amount of tryptophan that might be required for normal growth. Using graded levels of DL-tryptophan ranging from practically zero to 0.4%, it was found that pigs receiving 0.2% of DL-tryptophan in the diet showed as good a growth response as those receiving levels of 0.3 and 0.4% DL-tryptophan. Since levels above 0.2% tryptophan had no significant effect on the growth or development of the pigs, these studies show that 0.2% tryptophan is adquate for growth of the weanling pig. These findings should be of importance to the swine industry and to the manufacturers of protein supplements because corn is notably deficient in tryptophan.

Similar studies have been conducted with lysine. Pigs fed on synthetic diets deficient in lysine lost 2 pounds over a 28-day period while litter mate pigs receiving 2% of DL-lysine-HC1 in the diet gained 25 pounds. Studies to determine the minimum quantitative amount of DL-lysine required by the pig have not been complete, but at least these data have shown that lysine is required for pig development.

Methionine has been recognized as one of the amino acids which is low in soybean oil meal. Pigs which were fed on a semi-purified diet low in methionine and cystine gained only 0.05 to 0.14 pound covering a period of 28 days. Pigs of the same breeding, age and weight feeding on diets fortified with 0.6% of methionine gained at a normal rate ranging from 1.16 to 1.38 pounds. Since rat studies have shown that one-half of the methionine requirement may be replaced by cystine, it was thought advisable to try the same combination with swine. It was rather conclusively shown that the addition of 0.6% cystine to the basal diet gave very little growth response. However, when the diet was supplemented with 0.3% cystine and 0.3% methionine the growth rate was almost identical to the pigs that received 0.6% methionine. These results concur with those obtained on rats showing that approximately one-half of the methionine requirement of the pig can be replaced with cysine.

Even though adequate amounts of total protein were available in all the diets employed in these amino acid studies, the pigs failed to grow because of the lack of one of the essential amino acids. In all cases the addition of the missing amino acid to the pig's diet gave an immediate response proving that proteins are not stored and that the daily requirements of the pig must be met. It appears to me that when all of the amino acids that are essential for pig development and growth are determined, eventually protein supplements can be standardized on the basis of amino acid quality and thus reduce drastically the amount of total protein required for swine production. The foregoing statement is based on the premise that in rats and humans a perfect balance of amino acids in a diet reduces the total amount of protein required for normal development and growth.

"Animal Protein Factor"
No subject has attracted more attention among livestock producers,

meat packers and research workers than the so-called "Animal Protein Factor". The "Animal Protein Factor" derived its name from the fact that it was first found in association with products of animal origin. The "Animal Protein Factor" is not new to hogs. By supplementing hog diets with animal proteins we have been supplying in varying amounts the "Animal Protein Factor" and now we are gradually learning that one of the valuable contributions of animal proteins to swine rations was due to the presence of this factor which is essential for swine growth.

What is the "Animal Protein Factor"? The term "Animal Protein Factor" (APF) has been used to refer to a number of unidentified factors found in or associated with proteins from animal origin, but in general have been found lacking or low in vegetable protein supplements. One of the specific vitamins contained in the "APF complex" is vitamin B_{12}. It appears at present that there are several unknown factors in addition to B_{12} in the APF complex which are essential for properly nourishing the pig. Vitamin B_{12} is probably only one of the new vitamin factors in this complex which is furnished by proteins from animal origin.

All food products containing APF have not been identified. In fact we have hardly scratched the surface as to the potential source of APF. On the market now are many APF concentrates which are produced by bio-synthesis from waste products of certain antibiotics such as streptomycin, aureomycin, terramycin and many others. Some of the natural food products which have been shown to contain APF activity in varying amounts are milk products, liver and other glandular organs, meat products, fish products, egg yolk, cow maure and a host of other substances which are too numerous to mention.

The $64.00 question among most people is this: "Will APF replace all the animal protein supplement in swine rations?" The answer is not known. In my opinion we must not discard the use of animal protein supplements but we must continue to improve these animal by-products in the light of the new factors that are being discovered. To be sure I think we can fortify certain animal proteins that are low in APF, but we must not forget that animal proteins furnish other nutrients besides APF such as amino acids, minerals and probably many other nutrients which are unknown to man. The time is at hand when we will have to standardize all animal protein supplements not only on the basis of crude protein, but also on the basis of amino acid distribution, APF complex and other new factors.

Recent research at Purdue University has definitely shown that the APF complex is essential for the growth and development of swine in dry lot feeding. On a paired feeding experiment litter mate pigs receiving 1 mg. of vitamin B_{12} activity per 100 pounds of ration supplied by an APF concentrate (Merck and Company) were 44 pounds heavier at the same age than the control pigs with no supplementary source of APF. These pigs were fed in dry lot on a ration of vegetable origin composed of yellow corn, soybean oil meal, alfalfa meal, minerals, cod-liver oil and six synthetic B vitamins. Similar studies with pigs fed in a group comparison on approximately the same basal ration show that the addition of the "Animal Protein Factor" concentrate at a level of 2 mg. of B_{12} activity per 100 pounds of ration caused the pigs to weigh on an average 62 pounds more at the same age than the control group with no APF supplement. Feed efficiency was improved by the addition of the "Animal Protein

Factor." Pigs receiving no APF supplement required 398 pounds of feed per 100 pounds of gain while the group with the APF supplement required 382 pounds of feed. The differences in feed efficiency are not as great as should be expected by the increased growth rate in the APF group. However, since the efficiency of gains were compared when the control group weighed 160 pounds and the APF group 222 pounds it may be that the heavier hogs were fatter at the same age and since it requires more feed to produce a pound of fat, this may explain in part the small differences in the feed requirements. Other research at Purdue has shown that the degree of fatness in hogs is influenced more by weight than by age.

Outward symptoms of APF deficiency were characterized by smaller size, thin and unthrifty appearance and difficulty in breathing. A few of the pigs on the low APF ration showed specific symtoms of severe anemia which were indicated by low hemoglobin values and low red blood cell numbers. This concurs in part with research on humans which has indicated that vitamin B_{12} and other factors are essential for maintaining normal hemoglobin and red blood cell count.

Does green pasture contain the "Animal Protein Factor"? Research on this question has not progressed far enough to give a definite answer. Research at other experiment stations has shown that the response of pigs to APF supplements on pasture is not as great as the results obtained in dry lot. It is still an open question as to whether as great a "kick" can be obtained by the use of APF supplements if hogs are allowed to graze on lush green pasture and also when they are allowed to have access to soil. There is some data to indicate that there is a possibility that certain microorganisms in the soil may produce factors similar to APF. However, we do know this — that research at Purdue and many other experiment stations has shown time and time again that green pasture contains certain unknown nutrients that are not contained in harvested feeds. As yet man has not been able to put in the feed bag all of the nutrients that can be supplied by the same feeds plus green pastures.

Tankage
The nutritive value of tankage, meat scraps and other animal by-products vary considerably depending chiefly on the kinds of raw material which are used in producing these products. I think we must recognize that tankage and meat scraps produced in recent years have tended to be somewhat lower in nutritive value than similar products that were manufactured 20 to 25 years ago. This statement is based on the fact that early studies at Purdue University showed that a ration of corn and tankage with alfalfa meal and minerals in dry lot gave very good results in promoting growth and development of swine. However, a repetition of similar experiments in more recent years has shown that the same growth stimulation is not obtained from the use of tankage or meat and bone scraps as the only supplementary source of protein to corn in hog diets. Off hand it appears that the lower nutritive value of animal by-products is primarily due to the fact that smaller amounts of hearts, liver, kidney, beef trimmings and glandular materials are being used in making up the composition of tankage and meat scraps. Glandular tissues are much more abundant in vitamin B_{12} activity and also have a much better distribution of essential amino acids than blood, gristle or connective tissues.

Recently we completed an experiment at Purdue University in which

we were comparing the biological value of proteins for the growth of weanling pigs. All the pigs were fed semi-purified diets containing approximately 11% protein and experimental rations were designed so they were complete in all known factors, excepting the protein under study. It is rather interesting to note (Table 1) that the pigs fed on tankage (60%) as the only source of protein gained at a rate of 0.87 pound per day while the pigs on the same diet with dried skimmed milk as the source of protein gained 1.58 pounds daily. Three hundred and forty-one pounds of feed were required for 100 pounds of gain on the dried skimmed milk diet and 556 pounds of feed were required by the pigs receiving tankage. In another trial in which soybean oil meal was used the pigs gained 1.16 pounds daily. These data indicate that tankage lacks in either protein quality or some other factor for optimum pig growth when it is used in a semi-purified diet as the only source of protein. However, it should be pointed out that, no doubt, tankage would have a higher supplementary value when combined with corn in a natural ration for pigs. A summary of the results are given in Table 1.

We plan to continue studying the protein value of tankage and other meat by-products in order to identify what specific nutrients are lacking. In light of this knowledge, it appears that we should re-evaluate all meat by-products in order to more fully understand the change in nutritive value which apparently has been caused by the change in the raw products used in manufacturing.

Table I -- The effect of different sources of protein upon the daily gain and efficiency of utilization of protein.

Sources of Protein[1]	No. of Pigs	No. of Days	Average Daily Gain	Feed per 100 lbs. gain	P.E.[2]
Dried skim milk	4	70	1.44	376	2.42
Corn oil meal	4	70	1.13	347	2.62
Soybean oil meal	4	70	1.16	417	2.18
Dried skim milk	4	56	1.58	341	2.67
Corn oil meal	4	56	1.32	362	2.51
Tankage	4	56	0.87	556	1.63
Corn gluten meal	4	56	0.59	728	1.25

[1] Each ration contained 11 per cent protein with all of it coming from the test feed.
[2] Pounds gained per pound of protein consumed.

V

FAT IN HUMAN NUTRITION

M. I. Grossman
Department of Clinical Science
University of Illinois College of Medicine
Chicago

I. Proportion of fat in the diet

In considering the place of fat in human nutrition we seek answers to several questions:

1. How much fat is required in the diet?
2. How much fat do persons with free access to it select in the diet?
3. What is the optimal amount of fat in the diet?
4. What is the maximal capacity to digest and absorb fat?

How much fat is required in the diet? When rats are fed a fat free diet, they develop a characteristic deficiency disease with scaliness of the skin, retardation of growth, and other changes, all of which can be comple[te]ly reversed by feeding a highly unsaturated fatty acid-linoleic or linolenic acid. This indicates that, whereas most of the fatty acids found in the body can be synthesized from carbohydrate or protein precursors, the most un[-] saturated ones are "essential" articles of diet. Very few studies with fat free diets in humans have been done and no specific symptoms have been observed.

In human infants suffering from eczema it has been reported that th[e] blood level of unsaturated fatty acids is low and that feeding such acids al[-] leviates the disease. The essential fatty acids are widely distributed in bo[th] plant and animal foods so that the occurrence of dietary deficiency of ther[m] is unlikely.

In answer to our first question then, we may say that there is no min[-] imal requirement for fat for energy purposes and that suggestive but inad[e-] quate evidence has been obtained that unsaturated fatty acids are require[d] in the diet of man.

2. How much fat do persons with free access to it select in the diet? Fat is said to occupy a unique place in the dietary because of its ability t[o] impart richness of flavor, to change texture, and to add attractiveness to foods. It is said that low fat diets are monotonous.

When the fat content of the diet becomes reduced, as it did in Euro[-] pean countries during the World Wars, it is regarded as one of the most distressing of deprivations. Since, however, total caloric allowance was simultaneously reduced, it is difficult to evaluate this impression.

The statement is frequently made that "fat has high satiety value". This statement may be misleading if no further explanation is given. It is true that gram for gram, fat has more satiety value than other foodstuffs but calorie for calorie it does not. It should be recalled that in a beef-steak 1 gram of the fat has 8 times as many calories as 1 gram of the

meat; this is due to the high caloric value of fat plus the low water content of fatty tissue. Furthermore, we must consider the relationship between palatability and satiety value. It is well known that when the average individual is deprived of fat he diminishes his total caloric intake rather than stuffs himself with carbohydrates to make up the deficit. Thus, paradoxically, fat, because of its high palatability, increases caloric intake in spite of its high satiety value. It is interesting to note that in spite of the high desirability of fat, more if it is wasted in garbage than of any other edible foodstuff.

Because economic factors dictate the diets of large parts of the population, a high percentage of the calories is derived from the cheaper carbohydrates. When free choice of food is possible men doing heavy sustained work and athletes in training select a diet with 15 per cent of the calories from protein and the balance about evenly distributed between fat and carbohydrate. Obviously climate, local habits, and type of activity all play a role. The interesting observation has been made that with rising income there is a greater increase of fat than of protein in the diet.

In answer to our second question we may say that, within rather wide limits, fat and carbohydrate can be and are used interchangeably in the diet but most individuals consider a diet in which about the same number of calories is derived from fat as from carbohydrate to be a satisfying one.

3. What is the optimal amount of fat in the diet? Whenever one makes a statement about something being optimal one should always answer the question "optimal for what?". We have the answer in a few highly specific situations like the optimal amount of fat in the diet for prevention of "bends" at high altitude, or for performance of work in the Arctic. But we do not have answers to the important question of the optimal amount of fat for longevity, for prevention of arteriosclerosis, etc.

In human milk the calories provided by fat and carbohydrate are about equal.

The National Research Council recommends that 25 to 30 per cent of calories should be from fat.

4. What is the maximal capacity to absorb and digest fat? In normal subjects, fecal fat excretion does not increase as the amount of fat in the diet is increased isocalorically until more than 200 grams of fat per day is given.

II. Nutritional value of fats from various sources

Digestibility. As was just pointed out, in the normal range of fat content of the diet fecal fat excretion does not increase with dietary fat so that the ratio: $\frac{\text{fat ingested} - \text{fat excreted}}{\text{fat ingested}}$ increases as the amount of fat ingested increases. Therefore, when this ratio is used as a measure of "digestibility" there will be an apparent increase as the amount of fat in the diet increases. This can be explained by the fact that fecal fat in normal persons on normal diets is not unabsorbed fat but is fat that is secreted by the intestinal mucosa and continues to be present even on a fat free diet. Taking these facts into consideration, it may be concluded that all ordinary dietary fats including butter, meat fats, and vegetable fats and oils are relatively completely absorbed. As would be expected, fats with a melting point above body temperature are not well abosrbed. Hydrogenated oils, as long as their melting point does not go above 38° C., are as well abosrbed as the natural oils.

Much experimentation has been done on the relative nutritive value of butter fat and margarine. The results of these studies have been sumarized by the National Research Council as follows: When fortified margarine is u in place of butter as a source of fat in the mixed diet no nutritional differer ces can be observed, although important differences can be demonstrated i special restricted experimental diets. These differences are unimportant when a customary mixed diet is used.

Symptoms produced by feeding excess fat. At very high intake levels fats tend to produce diarrhea. At ordinary dietary levels only cocoa butter and goose fat produce diarrhea. Even at 150 grams per day lard and hydro-genated vegetable oil (HVO) do not produce diarrhea although beef fat is said to do so at this level.

It has been claimed that symptoms associated with dietary excess, such as nausea and abdominal fullness or pain, are more frequent in per-sons consuming a diet in which the predominant fat source is lard than in those using hydrogenated vegetable oil. In a recent extensive and carefully controlled study in our laboratory, Annegers and Ivy were unable to cor-relate any of these symptoms with either the level of fat in the diet (up to 150 grams, isocalorically) or the kind of fat (lard or HVO).

Carcinogenic action of heated fat. We have recently confirmed the fac that heating edible fats to temperatures somewhat above those used in fry-ing (350º) C. produces a weak carcinogenic substance, as detected by prod-uction of tumors in rats upon subcutaneous injection. However, we have not been able to confirm the report of Roffo from Buenos Aires who claimed that the feeding of heated fats to rats produced gastric cancer. We have nov maintained 7 generations of rats from Roffo's own strain on heated fat diet: for several years without observing a tumor. Incidentally, in these studies it was found that rats grew as well on heated as on unheated fat except when the level of fat in the diet was high which caused decreased food in-take in the animals on heated fat diets.

III. Digestion and absorption of fat
A. Theories of Fat Absorption

The classical theory of fat absorption states that neutral fat is hydro yzed by lipase to fatty acid and glycerol in the intestine, absorbed into the intestinal cell where it is resynthesized into neutral fat and then transferre to the lymph channels, by which route it reaches the blood stream. Bile salt play an important role by (1) reducing surface tension (they are wetting agents) thus helping to emulsify the fat and increase the surface for lipase action, (2) by acting as a coenzyme to lipase, and (3) by rendering the free fatty acids soluble in the slightly acid medium of the upper intestine. The question of whether the formation of phospholipid is involved in the resyn-thesis of fat in the intestinal cell is unsettled. However, a number of facts show a striking relationship between glucose absorption and fat absorption and, in the case of glucose, phosphorylation is considered to be a proven mechanism of absorption. In the disease sprue, both glucose and fat ab-sorption are impaired; and, experimentally, phlorhidzin, a phosphoryl-ation poison, decreases absorption of both, and feeding thyroid extract increases both. Giving phosphate with the foodstuff increases absorption of both. None of these effects are shared with amino acids.

Recently, the partition hypothesis of fat absorption, proposed by Frazer of England, has attracted wide attention. The essential points of

this theory are (a) that hydrolysis of neutral fats takes place to only a very limited extent, (b) the partial hydrolysis products, mono- and di-glycerides and fatty acids, together with bile salts form a highly effective emulsifying system, reducing the remainder of the neutral fat to an extremely fine emulsion, and (c) that the fine emulsion of neutral fat is absorbed without further splitting directly into the lymphatics whereas the small amount of fatty acid is absorbed into the portal blood stream, i.e., fat is partitioned between lymph and portal blood according to the state of hydrolysis. Frazer's evidence in support of this theory is all indirect, the main support being from two types of experiments: (1) Experiments purporting to show that fatty acid, unlike neutral fat, does not cause milkiness of lymphatics or rise in systemic blood fat but does cause rise in portal blood fat and is deposited in the liver. However, experiments by other workers are directly contradictory showing that when fatty acid is fed it appears in the lymph as neutral fat and fails to cause a rise in portal blood fat content. (2) Experiments purporting to show that mineral oil is absorbed in significant quantities when it is fed in the form of a very fine emulsion. It is widely recognized that traces of mineral oil, like other finely divided nonsoluble materials, may be abosrbed. But attempts to confirm Frazer's claim of absorption of mineral oil comparable to that occurring with fat, have, in general, not been successful. For instance, recent studies in our laboratory showed that the administration of very finely emulsified mineral oil to dogs with fistulas of the thoracic lymph duct did not lead to milkiness of the lymph nor to an increase in chemically detectible non-saponifiable material in the lymph.

Thus none of the evidence on which the partition theory rests can be considered to be established.

B. Special Physiological Mechanisms Assisting Fat Absorption and Digestion.

Fat is the most difficult of the food stuffs to digest. A number of special mechanisms operate to assist the process. Whenever there is a general reduction in digestive capacity, for example, as a result of removal of all or part of the stomach, of removal of a large part of the intestine, or of total or partial deprivation of bile or pancreatic juice from the intestine, it is the digestion of fat which is primarily, and sometimes solely, impaired.

The reservoir function of the stomach plays an important role in fat digestion, serving to send the chyme into the intestine at a rate at which it can be utilized. An adjunct to this reservoir function is the hormone enterogastrone formed by the upper intestinal mucosa when it is bathed with fat in concentrations above 10 per cent and acting to slow the emptying of the stomach. There has been some confusion concerning the concentration of fat required to decrease the rate of gastric emptying. About 10 per cent by wet weight is required and under some circumstances this value may not be attained even when fat represents a very high percentage of the total calories in the meal.

The importance of the gastric reservoir function in fat digestion is demonstrated by the fact that patients who have had one-half to three-fourths of their stomach removed for the treatment of peptic ulcer often show excessive fat excretion in the feces and failure to regain their preoperative body weight. We have recently shown that this steatorrhea can be partially corrected by feeding pancreatin containing lipase. This would indicate that a relative pancreatic insufficiency can result from the rapid intestinal passages that occurs after gastrectomy.

Other gastrointestinal hormones also assist in fat digestion. Thus fat in the upper intestine causes the release of cholecystokinin, a hormone which stimulates the gall bladder to contract and thus provides bile for fat digestion. Fat also serves as a stimulus for the release of secretin which stimulates the flow of pancreatic juice and pancreozymin which stimulates the secretion of enzymes by the pancreas.

VI

QUATERNARY AMMONIUM COMPOUNDS

L. A. Harriman
Chemical Research and Development Department
Armour and Company

For controlling microbial life, man has relied largely on heat, cold, and chemicals. The chemicals most widely used are chlorine, phenols, cresols, the salts of heavy metals, and more recently, sulfanamides, antibiotic agents, and quaternary ammonium compounds. It is interesting to note that the newest of these, the "quaternaries", are the only compounds which are competitive, or may become so, in one of the fields in which chlorine, one of the oldest antibacterial chemicals, is used. This is the field of sanitizing equipment and utensils used to prepare, process, and preserve food.

Chlorine was reported by Alock (1) in 1826 to have the power to arrest decay. In 1846, Semmelweiss used chlorinated lime to control puerperal septicemia without knowing what caused the malady, and was bitterly attacked for this innovation in hospital practice according to McCulloch (2). Today, chlorine is the most widely used antibacterial chemical.

Domagk (3) reported the antibacterial activity of quaternary ammonium compounds in 1935, almost a centry after the first use of chlorine as a germicide. Indicatory of the amount of work which has been done on these compounds, Dunn (4) in writing on their use in the food industry lists more than three hundred references.

Broadly speaking, the methods for testing "quaternaries" as germicides fall into two groups, (1) those which indicate the killing power under somewhat ideal, uniform conditions, useful for preliminary screening and (2) those which measure results in actual use or which introduce some of the factors which might be encountered under use conditions. The following material will relate some of the progress which has been made in the germicidal evaluation of these compounds, especially as shown in use test, or as affected by some of the factors encountered under operating conditions.

Electrical conductance of quaternary-tested bacteria. A new method of screen testing quaternary ammonium compounds is suggested by the work of Eggenberger and Harwood (5), which also offers an explanation for one of the mechanisms of killing. Measurements are made of the electrical conductance of bacterial suspensions to which "quaternaries" have been added. Figure 1 presents graphs of the apparent equivalent conductances of two different alkylammonium chlorides in the presence of heavy suspensions of viable Staphylococcus aureus cells. The upper curve, for "soya"-trimethylammonium chloride, shows an abrupt rise at the concentration where nearly complete kill (99.9+%) occurs. The rise in con-

centration of conductable solutes at the killing concentration implies es-
cape of cell contents through the cell wall and damage sufficient to cause
cell death. The smooth curve shows the equivalent conductance of hexyl-
ammonium chloride, which has little or no bactericidal activity and show
no evidence of cell-wall damage and escape of conductable substances.

Bacteriostasis. Bacteriostasis, hardness of water, and amount of organic
matter present are factors important in testing and using germicides. Ba
teriostasis was not considered in some of the early work with quaternary
ammonium compounds. In tubes of broth or plates of agar which are used
to determine whether exposure to the "quaternary" under test killed the
test culture, mere failure to grow was often mistaken for killing. Failure
to grown may be the result of bacteriostasis rather than killing, caused b
carry-over of the germicide from the point where it is applied to the or-
ganism. Today, all workers recognize the importance of taking measures
in test-work to eliminate stasis.

Table 1, from work by Eckfeldt and James, (6) shows the minimum
dilutions of five "quaternaries" and of phenol required to eliminate inhibi
tion of St. aureus and Eberthella typhosa. Note that some of the quaternar
ammonium compounds must be diluted a million times or more, whereas
dilutions of six- or eight-hundred times suffice to prevent phenol from in
terfering with growth of the organisms. Also, St. aureus is much more
sensitive to the static effect of "quaternaries" than E. typhosa, a differ-
ence not shown by phenol.

In test-work, neutralizers for the carried-over quaternary ammoni-
um compounds are used to eliminate bacteriostasis. It is important that
the neutralizer selected be known to be effective in neutralizing the parti
cular "quaternary" under test.

Although bacteriostasis complicates germicidal testing of "quater-
naries", it may be a definite benefit in actual practice. Such a case might
be in the sanitization of farm dairy equipment when drying does not
occur immediately. The extremely small quantities of "quaternaries"
would prevent growth of any organisms remaining in the residual mois-
ture.

Hardness of water. The effect of harness of water was studied by Arm-
bruster and Ridenour (7) by adding various cations and anions to solution:
of quaternary ammonium compounds under test. Figure 2 shows the effec
of four cations of 35 p. p. m. of a "quaternary". The rather low concentra
tion of the "quaternary" magnifies the effect of the ions. (The sanitizing
concentrations of most "quaternaries" range from 150 to 250 p. p. m.)
Calcium and magnesium ions at only 20 p. p. m. markedly decreased the
killing activity of the "quaternary", whereas sodium and potassium ions
had little effect. Figure 3 shows the effect of five anions. Phosphate and
carbonate ions at about 30 p. p. m. increased killing by the "quaternary",
whereas sulfate, nitrate, and chloride ions had little effect.

Armbruster and Ridenour (7) have also shown the effect of 100 p. p.
m. of calcium in a use test. Their method involved sanitizing artificially
contaminated drinking glasses in solutions of four different "quaternaries
Table 2 shows the counts obtained and the percentage kill. Concentrations
of 200 p. p. m. of three of the quaternary ammounium compounds gave a
of 99.9% in distilled water. No significant effect on this kill is caused by

100 p. p. m. of calcium at this concentration level, but a reduction in kill at the 100 p. p. m. level of "quaternary" is caused by 100 p. p. m. of calcium.

The fact that phosphate and carbonate ions increased the efficiency of "quaternaries" suggested to Armbruster and Ridenour (7) a way of preventing the interference of water hardness. To water with calcium, magnesium, and ferrous ions added to give the equivalent of 260 p. p. m. of soap hardness, various alkalies were added at a concentration of 500 p. p. m. The killing efficiency of alkyldimethylbenzylammonium chloride was then tested in this water. Table 3 shows that the presence of the alkaline correcting agents sodium bicarbonate, sodium tetraborate, trisodium phosphate, sodium carbonate, and sodium hydroxide raised the percentage kill of 100 p. p. m. of the "quaternary" from 95% to 99% or more. Increasing the concentration of the quaternary ammonium salt from 100 p. p. m. to 200 p. p. m. had an additional desirable effect.

The increase in pH values caused by the various alkalies was undoubtedly an important factor in correcting the adverse bacterial effects of the water hardness.

Presence of organic matter. Many workers have studied the influence of organic matter on the germicidal activity of "quaternaries". Table 4, from work by Eckfeldt and James, (6) shows that 10% blood serum, the equivalent of slightly less than 1% solid matter, greatly decreases killing on St. aureus and E. typhosa, whereas phenol is affected little or not at all. Indicative of the results in actual practice, Mueller and co-workers (8) reported that a concentration of 0.3% of cow manure or non-fat dry milk solids produced the first significant decrease in germicidal efficiency.

Johns (9) has contributed to our knowledge of the effect of added organic matter on quaternary ammonium compounds and hypochlorites. Using the glass-slide technique in which slides are artificially soiled with diluted milk containing the test organisms, he studied the killing efficiencies of four "quaternaries" and two hypochlorites. Table 5 shows a 99.5% kill in 5 seconds by three of the "quaternaries" at 100 and 200 p. p. m. concentrations. At these concentrations the hypochlorite killing time was between 12.5 and 20 seconds.

Work by Penniston and Hedrick (10) compares the sanitizing effects of a quaternary ammonium salt and a chlorine compound in the presence of the organic matter encountered in washing dirty eggs. Table 6 shows that both compounds, when used to sanitize dirty shell eggs, markedly reduced the count of egg pulp from the eggs; the reduction amounting to 99.9% for chlorine and 99.8% for the "quaternary". Penniston and Hedrick also report that the "quaternary" solution sanitized from 6 to 10 times as many eggs as the chlorine solution.

Guiteras and Shapiro (11) have proposed using "quaternary" sanitizers in the same water which is used for cleaning utensils. Their test-work was done on artificially soiled and contaminated glass slides. Table 7 shows the composition of two sanitizing detergent mixtures which they tested. In solution at a concentration of one ounce per gallon, both formulas sterilized the slides in 4 to 5 minutes, whereas the same formulas without the "quaternaries" permitted approximately 2.0% and 33.0% survival of the test culture. The sanitizing concentration of "quaternary" A was 250 p. p. m.; of "quaternary" B, 500 p. p. m.

Table 8 shows the results of tests run by Kahlenberg (12) on the stability of a germicidal detergent in the wash water of a mechanical egg washer, the "quaternary" salt being "soya"-trimethylammonium chloride. The figures shown represent colonies from 0.01 ml. of the wash water. After eight hours of use no significant increases in counts occurred on 5 of the 6 days of operation, and the increases on the remaining day do not indicate depletion of the quaternary ammonium chloride.

Toxicity. Most "quaternaries" are entirely free of toxic effects when used in concentrations necessary for sanitizing.

Under date of April 27, 1949, the Meat Inspection Division of the Bureau of Animal Industry issued Memorandum No. 123. This memorandum states that "Aqueous solutions of quaternary ammonium compounds, sodium hypochlorite or chloramine may be used as sanitizing agents and mold inhibitors on equipment, floors, walls and ceilings of edible product departments of official establishments in accordance with" certain directions for their use.

Mildness. The mildness of the quaternary ammonium compounds is well known. In sterilizing concentrations, they are markedly low in corrosive action on metals especially when used in the presence of inorganic salts such as tetrasodium pyrophosphate or sodium carbonate. As a group, they are non-irritating to the skin.

Cost. Quaternary ammonium compounds are somewhat costly as sanitizers. Foster (13) states that a value of 0.8 cent per gallon would be representative for cost estimates, whereas hypochlorites may be estimated at 0.2 cent. However, the possibility of using "quaternaries" in combination with alkaline detergents offers the possibility of decreasing sanitizing costs by cleansing and sanitization in one operation.

Summary. Although no attempt has been made to compare quaternary ammonium compounds with hypochlorites in all respects, the tabulation in Chart A lists advantages of each. The differences between the two classes of compounds suggest different fields of use.

Chart A. Some qualities of chlorine and "quaternary" sanitizers.

Chlorine	"Quaternary"
Low in cost	Non-corrosive
Resistant to water hardness	Non-irritating
Leaves volatile residue	Resistant to organic matter
	Stable

It is probable that cost alone will remain, for some time to come, the most important factor in the choice between chlorine and quaternary ammonium salts. On the other hand, where stability and non-corrosive, non-irritating properties are required, the "quaternaries" merit consideration.

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Table I -- Minimum Dilution of Five "Quaternary" Disinfectants and
Phenol Required to Eliminate Bacteriostasis. (Eckfeldt and
James, Soap-Sanit. Chemicals 23 (11) 125 (1947).

Test culture	Germicide	In broth	In agar
S. aureus	Quat. A	100,000	150,000
	Quat. B	800,000	1,200,000
	Quat. C	1,000,000	700,000
	Quat. D	1,000,000	1,000,000
	Quat. E	800,000	1,200,000
	Phenol	600	600
E. typhosa	Quat. A	50,000	25,000
	Quat. B	80,000	80,000
	Quat. C	50,000	5,000
	Quat. D	100,000	50,000
	Quat. E	120,000	12,000
	Phenol	600	800

Table II -- Bactericidal Action of Four "Quaternaries" With and Without Added Calcium. Staph. aureus, 15 seconds exposure, 20°C. Armbruster & Ridenour, Soap-Sanit. Chemicals, 25 (7) 104 (1949).

Test	p. p. m.	A Av. count per glass	A % kill	B Av. count per glass	B % kill	C Av. count per glass	C % kill	D Av. count per glass	D % kill
Dist. water	None	40,000		17,500		20,200		11,000	
	100	410	99.0	180	99.0	1,400	93.1	292	97.3
	150	45	99.9	60	99.7	650	96.8	16	99.9
	200	30	99.9	3	99.9	730	96.4	10	99.9
Ca, 100 p. p. m.	None	13,000		16,100		19,300		14,500	
	100	580	95.5	425	97.4	6,900	64	1,400	90
	200	4	99.9	9	99.9	1,200	91.4	63	99.6

A. alkyldimethylbenzylammonium chloride

B. para-di-isobutylethoxydimethylbenzylammonium chloride

C. (acylcolaminoformylmethyl)pyridinium chloride

D. alkyldimethylethylammonium bromide

39

Table III — Effect of Various Hard-Water-Correcting Agents Used with Alkyldimethylbenzylammonium Chloride. Staph. aureus, 15 seconds exposure, 20°C. Armbruster & Ridenour, Soap-Sanit. Chemicals, 25, (7) 105 (1949).

Correcting agent	pH of sanitizing solution	% kill 100 p. p. m. sanitizer	% kill 200 p. p. m. sanitizer
Sodium hexametaphosphate	5.0	Under 50	Under 50
Sodium pyrophosphate	7.1	95	99.9
Sodium bicarbonate	7.4	99	99.9
Sodium tetraborate	8.2	99.2	99.9
Trisodium phosphate	9.1	99.4	99.9
Sodium carbonate	9.5	99.5	99.9
Sodium hydroxide control	8.0	99.1	99.9
Sodium hydroxide control	9.0	99.3	99.9
None	7.0	95	99.9
In dist. H_2O		99	

Table IV -- Effect on Counts of Adding 10% Blood Serum to a "Quaternary" and to Phenol. Eckfeldt & James, From Tables No. 8 and 9 Soap-Sanit. Chemicals, 23, (12) 157 (1947).

	S. aureus		E. typhosa	
	Without serum	10% serum	Without serum	10% serum
Quaternary C	7500	2500	7500	1000
Phenol	60	50	90	90

Table V -- Time Required by Four "Quaternaries" and Two Hypochlorites to Kill Staph. aureus at 20°C. Johns, Can. J. Research, Sec. F, 25, 79 (1947)

Germicide	Time in sec. for 99.5% kill		
	200 p. p. m.	100 p. p. m.	50 p. p. m.
Quat. A	5	5	10
Quat. B	5	5	10
Quat. C	5	5	10
Quat. D	15	15	20
Na hypoch. A	12.5	15	
Na hypoch. B	20	20	

A. alkyldimethylbenzylammonium chloride
B. alkyldimethylbenzylammonium chloride
C. (diisobutylphenoxyethoxyethyl)dimethylbenzylammonium chloride
D. (acylcolaminoformylmethyl)pyridinium chloride

Table VI -- Effect on Egg Pulp of Washing Shell Eggs with Chlorine or a "Quaternary." Penniston and Hedrick, Food Technol., 1, 241 (1947)

Plate count per g. of egg pulp		
Water	Cl, 100 p. p. m.	Quat., 0.04%
1,430,000	1,490	3,040

Quat., (acylcolaminoformylmethyl)pyridinium chloride

Table VII — Composition of Detergent Sanitizers. Guiteras and Shapiro, J. Bact. 52, 635 and 636 (1946).

	Formula A	Formula B
Trisodium phosphate	33 g.	50 g.
Sodium carbonate	33	25
Borax	33	--
Tetrasodium pyrophosphate	--	25
Quat.	3	--
Sanitizer	--	6
Nonionic wetting agent	1.5	--
pH	11.5	10.0

Quat.   -   cetyldimethylethylammonium bromide
Sanitizer -   cetyldimethylethylammonium bromide plus alkylated arylpolyether alcohol

Table VIII -- Plate Counts of Wash Water with a Sanitizing Detergent in an Egg Washer. "Soya"-trimethylammonium Chloride. (Kahlenberg, unpublished work.)

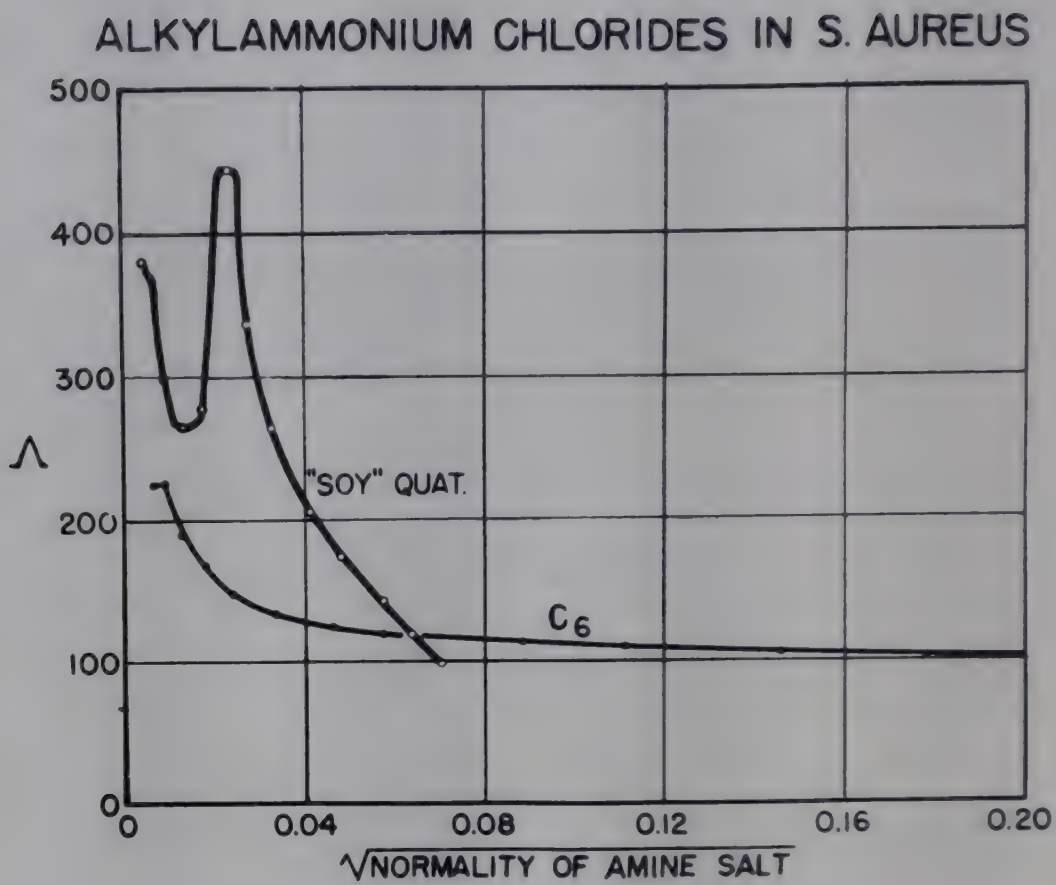|	Colonies per plate				
Hr. in use	Mon.	Tues.	Wed.	Thur.	Fri.
2	2	2	2	0	4
4	0	19	4	3	3
6	7	23	2	4	14
8	8	30	3	0	6

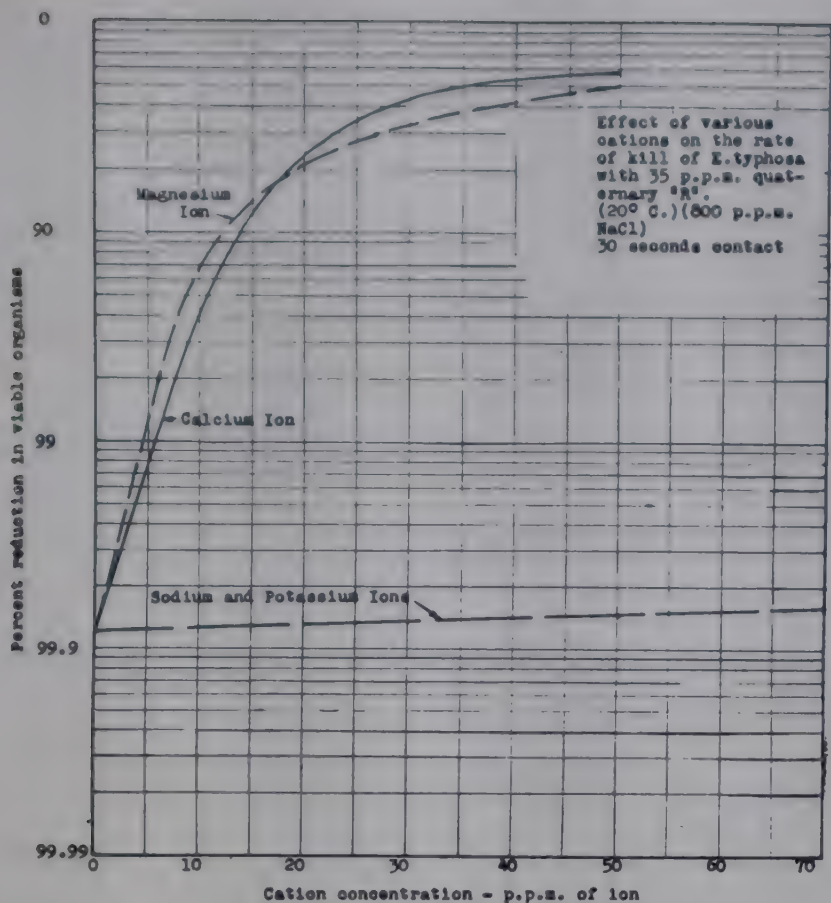Figure 1. (Eggenberger and Harwood, loc. cit.)

Figure 2. Armbruster and Ridenour, Soap Sanit. Chemicals
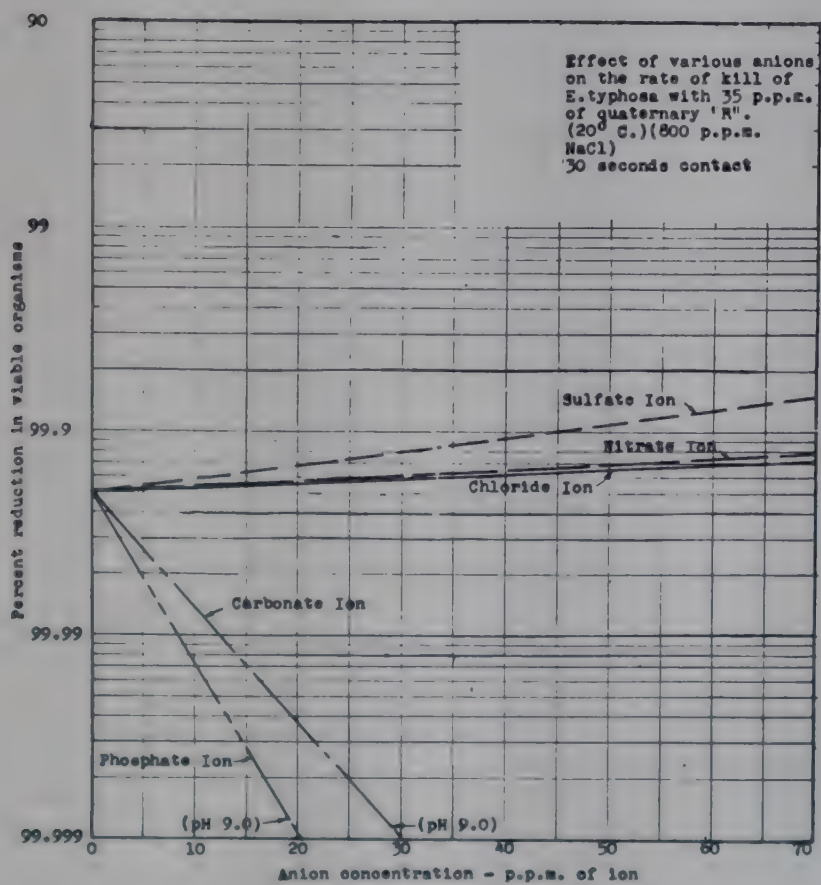25 (7) 104 (1949).

Figure 3. Armbruster and Ridenour, Soap Sanit.
Chemicals 25 (7) 104 (1949).

43

VII

THERMAL RESISTANCE OF BACTERIAL SPORES

H. Sugiyama and G. M. Dack
Department of Bacteriology and Parasitology and the
Food Research Institute, the University of Chicago

. Spores of microorganisms constitue an important cause of food spoilage and therefore are of great concern to food processors. Often in an attempt to control spores in processing a food, the quality of the product is seriously impaired and no longer resembles the fresh state. More fundamental knowledge of spore formation and heat resistance of spores is needed before improvements can be made in processing foods.

Since the recognition of bacterial spore by Ferdinand Cohn in 1876, numerous and extensive studies have been made concerning the practical and theoretical importance of their thermal resistance. There is still much work to be done before the heat tolerance of spores can be explained. In this short review we are attempting to summarize the general types of experiments representing the direction which research has taken in this field, as well as the theories that have been advanced to explain the phenomenon of the high heat resistance of bacterial spores.

The importance of the sporulating medium in the heat resistance of the developing spores has been amply verified. In the case of Bacillus subtilis it is the type of peptone rather than its concentration that is of significance. Favorable or unfavorable cultural conditions per se are not the determining factors in thermal resistance. Thus, only meager growth occurs in isoelectric gelatin but extremely resistant spores are produced, while in peptone-water plus bile growth is just as sparse but the spores are only moderately heat resistant (Williams, 1929).

It is extremely difficult with some spore-formers, including Clostridium botulinum, to produce yields of spores of heat resistances such that one batch will have the same heat resistance as another. Esty and Meyer (1922) have stated: "spores generated in different flasks of the same medium, inoculated with equal amounts of the same stock culture and incubated for the same period, may show striking differences in heat resistance." This difficulty of repeating experimental results is a serious problem in research with these organisms. The development of a sporulating medium of known composition and information on the mechanism of spore formation may offer a partial solution to the difficulty. Significant contributions in this field

have been made in recent years (Foster and Heiligman, 1949).

The temperature of incubation during the formation of spores affects their heat resistance. The thermal tolerance of spores of the genus Bacillus can be correlated in general with growth temperatures (Lamanna, 1942). The thermal tolerance of the spores of bacteria isolated from evaporated milk is maximum at the optimum growth temperature, the resistances falling off when sporulation occurs on either side of the optimum. Several subcultures at a given temperature are needed before the full effect of the temperature becomes apparent in the heat resistance of the spores (Theophilus and Hammer, 1938). The effect of the temperature of sporulation on the thermal resistance has also been shown for B. subtilis (Williams, 1929), B. anthracis (Weil, 1899), and C. botulinum (Sugiyama, unpublished observations).

The heat resistance of a spore suspension is subject to change with the conditions of aging. B. mycoides spores develop their highest resistance under conditions of moderate temperature and moisture, the maximum probably being reached by the sixtieth day (Magoon, 1926). Spores subjected to continuous drying or alternate drying and wetting become slightly more heat resistant than those held the same length of time in water (Curran, 1935). On the other hand, different lots of C. botulinum spores stored in their own liquor of growth in the icebox behave differently; some maintain the same degree of thermal resistance for 4 1/2 months, others lose part of it within two weeks (Sommer, 1930). Rapid drying in vacuo of C. botulinum spores decreases the heat resistance slightly but it remains constant for at least 347 days whether the spores are kept in the icebox, at room temperature or at 37° C. (Esty and Meyer, 1922).

In cultures, the time of appearance of spores of maximum heat resistance varies with the culture media and the temperature of incubation. The maximum heat resistance of spores of C. botulinum is developed at 37° C. in 1 1/2 to 3 1/2 days in pea-gelatin, 2 1/2 days in pea-peptic digest, and 6 to 10 days in brain medium. At 28° C. incubation, the maximum resistance in pea-gelatin is reached in 3 1/2 days, and in peptic digest and brain media in 8 days. It has been suggested that young spores, probably of the first generation, are the most heat resistant (Esty and Meyer, 1922).

The mechanics of harvesting the spores may result in spore suspensions of different thermal tolerances. The spores can be separated into fractions of different maximum resistances by means of the supercentrifuge, the most resistant spores being thrown down in the first operation. Successive recentrifuging of the "effluents" give spores of progressively lower heat resistances (Yesair and Cameron, 1936). The removal of clumps of spores, e.g., by filtration, can alter the heat survival values (Williams, 1929).

The nature and pH of the medium in which the spores are heat processed markedly influences the thermal resistance. C. botulinum spores are most heat resistant when suspended in phosphate buffer with a pH between 6.3 and 6.9. The effect of pH becomes marked when it is below 5.0 and above 10 in double strength veal infusion broth (Esty and Meyer, 1922). C. perfringens spores are most heat tolerant at pH 5.0 in Clark and Lub's buffer, but in peptone the optimum pH is 6.6 (Headlee, 1931).

Concentrations of sodium chloride up to 4% increase the time necessary to heat-kill spores of aerobic organisms in canned pea liquor (Viljoen, 1926). C. botulinum spores are less susceptible to heat processing

45

when suspended in 0.5-1.0% saline; between 2-6% there is no apparent effect; but beyond 8% the resistance is greatly decreased (Esty and Meyer, 1922).

Concentrated solutions of sugars are known to have a protective action on bacteria that are being heated. The protective action of dextrose and sucrose on Escherichia coli increases with the osmotic pressure in a series of increasing concentrations of sugars. Equimolar solutions of different sugars, do not, however, show the same degree of protection; sucrose is very effective, but maltose and lactose give little protection from heat. Washing the cells after exposure to the hypertonic solution removes the effect. Within limits, the longer the time of contact between the sugar and the organisms, the greater the effect. The addition of the sugar after the heat treatment does not enhance the survival of the heated organisms (Fay, 1934). The same phenomenon has been observed with the spores of the hay bacillus, turbidity measurements indicating that the bacteria become partly dehydrated in concentrated solutions (K. von Angerer and Kuester, 1939). Similar observations have been made with C. botulinum spores. In this case, however, the heat resistance reaches a maximum within a few minutes' contact with 50% sucrose and then remains so for many hours (Sugiyama, unpublished observations).

The mechanism of action of meat curing agents in the heat resistance of the spores is disputed. According to one point of view, spores may be killed at lower temperatures when curing agents are present. (Jensen, 1945). However, in the case of the putrefactive organism P. A. 3679 the curing agents were not found to influence the heat processes necesary to sterilize meat inoculated with the spores (Stumbo et al., 1945). It may be that the curing agents exert their influence by inhibiting the growth of the spores surviving the heat processing (Yesair and Cameron, 1942).

The heat resistance of spores is markedly increased when they are suspended in oily material. C. botulinum spores have been found to have an increased survival during heating in fish products with oil (Lang and Dean, 1934). Moist or dry micrococci are of low resistance when heated in a moist environment. The presence of moist fat increases this resistance while a condition of dry sterilization is approached when dry micrococci were heated in dry fat (Yesair, Bohrer and Cameron, 1946).

In thermal resistance tests the heat processed spores must be cultured to determine their viability. The conditions of testing this viability may have a profound effect, especially when the heated spores undergo a long period of dormancy which is not included in the incubation of the recovery cultures. It is evident that these conditions do not influence the actual heat resistances of the spores but that the effect is only an apparent one. This dormancy is not an inherent property of the spores but is an environmental influence (Morrison and Rettger, 1930; Foster and Wynne, 1948); however, cell injury is probably a contributing factor to delayed spore germination (Stumbo, 1949). The temperature of incubation of the recovery cultures has also been shown to influence heat resistance values. C. botulinum spores were found to have greater heat survival values when recovery cultures were held at 24° C. and 27° C., as compared to 31° C. and 37° C. (Williams and Reed, 1942).

Some of the earlier work on increasing the thermal resistance of spores by selection may be open to criticism (Rahn, 1932). Experiments which are not open to these objections show that spores of Bacillus glo-

bigii can be selected for increased heat resistance (Davis and Williams, 1948).

The kinetics of heat destruction have been studied extensively. The order of death of heated spores is usually logarithmic — i.e., a plot of the logarithm of the survivors against the intervals of heating time gives a straight line. From the very nature of this graph it is clear that the greater the number of spores per unit volume, the higher the thermal death tin e values. However, the death curve is not linear throughout, there usually being a few spores which are of a comparatively much greater heat resistance. As previously indicated, the thermal tolerance of all the individuals in a spore population is not uniform, since a spore suspension can be separated into fractions of different maximum resistances by means of the super-centrifuge (Yesair and Cameron, 1936).

Because the death of the spores can be described logarithmically does not necessarily mean that a monomolecular reaction in the chemical sense is operating; the graph can be merely a description of the process. The literal interpretation of the logarithmic order of death as a monomolecular reaction seems untenable since this involves the assumption that the organisms are essentially uniform in resistance. In the case of the death of bacteria by disinfectants, two alternative explanations have been offered. One hypothesis is that since the varying resistance of the spores in any given suspension can be described in the form of a frequency curve, the survival curves may be simply an expression of this difference in resistance. This would imply that the heat resistance distribution curve would have to be extremely skewed. In the case of disinfectants, however, the time-survivor curve yields an approximately normal distribution if the survival times are plotted as logarithms instead of absolute numbers (Withell, 1942). The other hypothesis is that the death of any given spore during any given interval of time is determined by a multitude of small and independent causes — "by chance" in the statistical sense. "If the chance of each bacterium dying during any unit of time is x, and remains x over the whole period of the experiment, then the death rate will be the same during each unit of time; the survivors at the end of any one time interval will suffer the same proportionate decrease in their numbers during the time interval which follows, and a logarithmic curve of decrease will result." (Topley and Wilson, 1946).

Whatever may be the true explanation of the logarithmic order of death, the study of the kinetics of the death process leads to a deduction of fundamental importance. The high temperature coefficients of heat sterilization, calculated from the death rate constants obtained from heating spores at different temperatures, can best be explained on the basis of protein denaturation (Rahn, 1945).

A brief summary of some of the experimental work on the heat resistance of spores has been presented; now, some of the theories on the nature of this heat resistance will be given. The insulation of the cell content by the spore wall does not seem to be the explanation since it has been calculated that to explain the resistances observed, the spore wall would have to have an insulating capacity about a million times greater than that of air (Virtanen, 1934). Furthermore, mere insulation would not account for resistances of 440 minutes at 100° C. but only 5 minutes at 120° C. (Rahn, 1945). However, spores which shed the exine of the spore coat upon germination are more heat resistant than those which absorb it (Knaysi, 1938).

Moreover, bacteriophage in spores is more resistant to heat than the same bacteriophage in culture filtrates. Thus, although the phage itself may be destroyed at 60° C. for ten minutes, phage present in the spores can resist heating at 90° C. for ten minutes, this time and temperatures approximating the maximum heat resistance of the spores themselves (Cowles, 1931).

The temperature at which protein coagulation occurs is roughly inversely proportional to the water content (Lewith, 1890). The ineffectiveness of dry heat sterilizaaion as compared to moist heat is well known. The enhanced resistance of organisms suspended in certain sugar solution is due to a partial dehydration of the organisms (K. von Angerer and Kuester, 1939). Contrary to the conclusions of earlier workers, recent determinations demonstrate that vegetative cells and spores have approximately the same amount of moisture (Virtanen and Pulkki, 1933; Henry and Friedman, 1937). However, the distribution of this water in the spores and vegetative cells seems to be different, there being a greater percentage of bound water in spores than in vegetative cells (Friedman and Henry, 1938). The nature of bound water is such that it will not participate in freezing or chemical reactions (Gortner, 1949; Bull, 1943). If this observation can be verified one of the basic reasons for the high heat resistance of bacterial spores will have been determined. It is of interest to note that there is no apparent correlation between the heat tolerance of vegetative cells and the spores of the same species (Zimmerman, 1947).

Since the salt concentration determines the amount of coagulated albumin that can be obtained from serum, it has been suggested that the low ash content of cells grown in medium of low salt content may be of prime importance in thermal resistance (Williams, 1929). The experimental observation that B. subtilis spores grown in isoelectric gelatin (a medium of low ash content) have high heat resistance cannot be disputed; however, the interpretation may be questioned. The action of heat on proteins is not merely coagulation; the primary reaction is that of denaturation followed by coagulation under suitable conditions, among which is the presence of salts. It would seem that the more important part of the reaction is that of denaturation which may not be affected by salt concentrations (Neurath et al., 1944).

Spectrochemical analyses show that the spores have a higher content of calcium, copper and manganese than the corresponding vegetative cells; the converse exists with potassium. Even among the non-spore forming organisms, the thermophiles have a higher calcium content than those species having a lower optimum temperature of growth (Curran, Brunstetter and Myers, 1943). The exact significance of these differences is not known.

It has been argued that a logarithmic order of death excludes the inactivation of enzymes as the cause of death, since the destruction of just a few of the many enzyme molecules would not be expected to kill the organism (Rahn, 1943). This argument is no longer valid since recent calculations indicate that certain enzymes, possibly associated with genes or cellular synthesis must exist in concentrations of only one or a few molecules per cell (McIlwain, 1946). Many of the enzymes of the spores may be in an inactive and resistant state (Virtanen, 1934). How these inactive enzymes may have become resistant to heat is not made clear. The denaturation of one vitally essential gene which has been postulated to ex-

48

plain the logarithmic order of death (Rahn, 1943) is not the ultimate answer, since this would still pose the question as to why the gene in the spore is more thermostable than in the vegetative cell.

The spores of C. botulinum grown in a medium containing oleic acid have greater heat tolerance than those grown in the same medium without oleic acid. The removal of the fatty acid by treatment of the spore suspension with chloroform and petroleum ether does not decrease the high heat resistance of the spores grown in the oleic acid containing medium. Nor does the addition of oleic acid to the spore suspension obtained from the control medium enhance the heat resistance of these spores (Sugiyama, unpublished observations). Thus the fatty acid may have been incorporated into the spore. Fat extraction of spores with chloral hydrate and trichloroethylene reduces the heat resistance along with the ability to be stained by the acid-fast technic (R. von Angerer, 1939). The chemical composition of a bacterial cell is related to the chemical composition of culture medium (Porter, 1946). The heat denaturation of certain proteins like serum albumin is retarded in the presence of fatty acids, the longer the hydrocarbon chain of the fatty acid, the greater being the protection conferred on the protein (Boyer et al., 1946 and 1947). Thus, if the heat denaturation of some essential protein or proteins is the factor limiting the heat tolerance of the spores, and this protein can be protected from heat denaturation by fatty acids, then the incorporation of a greater amount of fatty acid into the spore cytoplasm may increase the heat resistance of the spores. This assumption is made on the basis that the fatty acid maintains its integrity in the cell. It has not been demonstrated, however, that this is the case, and it may well be that the fatty acid is broken down in the metabolism of the cell.

Summarizing, one can only say that the mechanism whereby bacterial spores are able to withstand drastic heat treatment is still not definitely known. The heat tolerance is probably due to many factors, one factor being the limiting one under certain experimental conditions, and another the limiting one under a different set of conditions. However, there is general agreement that the chemical composition of the spore must be different from that of the vegetative cells from which they originated.

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VIII

FACTORS AFFECTING BACTERIAL SPORE GERMINATION

H. O. Halvorson
Department of Bacteriology
University of Illinois

In the growth of bacteria, there are no phenomena that are as poorly understood as the ones that govern the formation and germination of spores. My comments will be confined to the latter process.

Most bacteriologists are satisfied with the statement that spores germinate when they are placed in a favorable environment. This is merely stating a fact, and does not explain what happens or the factors that govern. It presumes, furthermore, a knowledge of what constitutes a favorable environment, but unfortunately this is not true. As a matter of fact, our knowledge concerning the factors that govern spore germination is so limited that I hesitated in sonsenting to prepare a manuscript on this topic.

I consented finally only because I felt such a discussion might stimulate an interest in some of the problems which are involved, and that such interest might result in more active research in this field. This discussion, therefore, is not intended as a critical review of the literature or a report of new research.

A better understanding of the process is of considerable importance, both from the academic and the practical point of view. What are the factors involved that will allow a dormant cell suddenly to start imbibing water, set in motion the enzymes, and otherwise take on the activities of a vigorously growing cell, when under conditions that appear very similar, the cell would remain inactive, and apparently dormant? The answers to these questions would certainly be exciting as well as useful. The industrial microbiologist would profit immensely by a better understanding of the factors that govern spore germination. He might then be able to control the process so as to have the spores disappear to form vegetative cells that could be easily killed, or perhaps he could control conditions to such an extent that the spores would be prevented from ever germinating in products that he wanted to preserve.

Our lack of knowledge is due in part to inadequate tools for a careful study of the process. Up to the present time, relatively few techniques have been introduced for the study of this problem. Most of these techniques are rather inadequate in some important respects.

The earlier bacteriologists studied spore germination through microscopic observation. For this purpose they would make wet preparations and would examine them under the microscope at frequent intervals to see when germination had taken place. As a criterion, they assumed that the end point of germination had occurred when the newly formed vegetative cell was about ready to divide. By this method the investigator could study
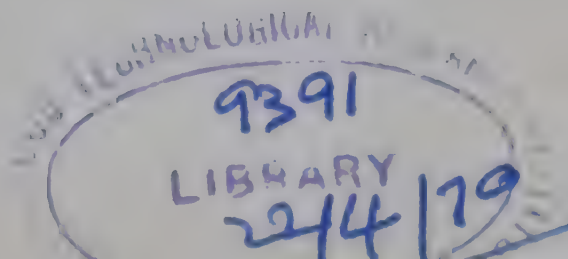
the individual spores in a preparation to see how many would germinate and how long it would take. Those spores that failed to germinate in accordance with this criterion in a reasonable length of time were assumed to be dead spores. While this technique does give some useful information it is so time-consuming and tedious that not many factors can be studied. It is also unlikely that all of the spores that fail to germinate in a reasonable length of time are dead. Under slightly different conditions, they might germinate in a much shorter time.

Another technique that has been and still is used is to follow the decrease in spore population by observing the change in the number of cells that can withstand heating to a terperature that will kill vegetative cells. This technique is unsatisfactory because slight changes in the early stages of the spore may materially alter its heat resistance so that it would be killed before it has become a vegetative cell. Another objection is that some of the newly formed vegetative cells may produce new spores during the course of the experiment. This was amply illustrated in a relatively simple experiment we performed this past year. A quantity of spores was inoculated into a sterile medium and then at varying intervals of time during incubation, aliquot samples were removed and heated to a temperature of about 105° C. for a period of ten minutes. Following the heating, the spore population was determined by a dilution count in a medium in which it was known that the spores would germinate. The results showed that during the first 24 hours there was a continual drop in the spore population to about one half of the original value. Following that time, however, the spore population increased until it finally reached a population equivalent to the starting population, and from there on it fluctuated over relatively narrow limits for a long period of time. It was obvious from these results that some of the spores had formed vegetative cells, which in turn began to produce new spores before all of the old ones had germinated, and therefore, at later time intervals, it was impossible to determine how many of the old spores had germinated and had been replaced by new ones. This technique is, therefore, a very unsatisfactory one to use if one wishes to study the factors that influence spore germination.

Another technique that has been used is to study various kinds of media, as sub-culture media, in determining spore counts. This may be done by inoculatin the spore suspension into a solid medium so that as the spores germinate they will produce discreet colonies which can be counted, or the spores may be counted by inoculating liquid media, determining the population by a dilution count. When this is done it is found that some media will allow more of the spores to germinate than others, and therefore, higher counts are obtained. It is presumed, therefore, that the medium which gives the highest count will be the one which is most favorablc, and perhaps one that will allow all of the spores to germinate. This can then be considered the control count. When the counts obtained on other media are then compared with this, one can get information dealing with the effect of various factors upon the process of germination. This is the type of technique that has been used in most recent studies.

In a few instances, single-celled isolations of spores have been made, which have then been transferred to suitable media, and observations are then made on the length of time required for germination as

evidenced by the appearance of turbidity. While this may be a very fruitful technique, its usefulness is impaired by the large amount of work involved.

The cytological changes that take place during germination are not particularly revealing. If one observes spore germination under the microscope, he may observe three main types. The first stage is much the same in all types. The spore becomes distended and the refractory appearance disappears. The first type appears to be very simple. The spore gradually acquires the normal dimensions of the vegetative form which then divides by fission. In the second type, the spore capsule opens at one of its poles, so that the diredtion taken by the growing bacillus is in a line with its length. The final expulsion of the new cell is effected by the spore membrane, the tension of which is gradually increased to such an extent that it forces out the newly formed begetative cell. In the third type, the spore membrane does not open up at the poles, but instead in a line corresponding to the equator of the spore. The shell may not rupture all the way around, but the two halves may still remain attached at one point. A portion of the new bacillus is then pushed out of the spore membrane, while it continues to elongate and at the same time bends through a ninety degree angle. One extremity is finally pulled out of the opening in the outer wall of the spore while the other extremity remains attached to the spore.

According to Robinow (1), spores contain discreet nuclei, and these are found in the periphery of the cells. During germination, according to him, after a short period of incubation in nutrient solutions, the nucleus leaves the periphery of the cell and enters the cytoplasm. The basophilic character of the cytoplasm increases markely, and the outlines of a nucleus become indistinct. However, within a short time, distinct chromatinic bodies again appear. According to him, therefore, the first change that takes place during spore germination is a change in the nuclear material, or rather a change in the distribution of the nuclear material.

In general, the factors that favor germination are those factors which are also favorable for the growth of the vegetative cell. Thus we find that the most favorable temperature range for the germination of spores will be one within the growth range of the organism. However, it has been found that germination generally is more sensitive to changes in temperature than is growth of the vegetative cell, although this may vary considerably with the species of organism involved. Thus, for example, Bacillus anthracis will not germinate at room temperature, the minimum appearing to be in the region of 35 to 37° C. Bacillus megatherium, on the other hand, will germinate very well at temperatures from 20 to 25° C. The spores of Bacillus mycoides can germinate from temperatures between 8 and 53° C.

Water, of course, is an essential constituent for germination, since the initial stage in the process is one in which water is imbibed from the outside medium. However, very little is known about the limiting concentrations that are necessary for germination. This is not to be wondered at, since we do not even have adequate information on the limiting concentrations that are necessary for growth.

Germination is influenced to some extent by the osmotic pressure produced by various salts and other compounds. In general, the processes that initiate germination are more sensitive to these changes than the vegetative cells. However, here, as in the case of growing cells, it is sometimes

53

difficult to differentiate between osmotic effects and toxic effects of the chemicals which are used to produce the osmotic pressure.

Germination is markedly influenced by the presence or absence of exygen, depending upon the type of organism involved. Aerobic organisms will germinate only if adequate supplies of oxygen are present. The reverse is true for the anaerobes. With these, oxygen will interfere with germination more effectively than it will with the growth of vegetative cells. The early bacteriologists, who studied the anaerobic spore forming organisms, generally agreed that the spores are unable to germinate until a suitable low oxidation-reduction potential is established in the medium. The lower the potential the shorter is the lag period for germination. Thus Miss Stephenson (2) reports in her book on the metabolism of bacteria that in the case of Clostridium tetani, the shortest germination time was found to be four hours when the potential was -0.05 volts or more negative; at an E_h value of +0.10 the lag was eight to ten hours; and at an E_h of +0.11 volts at pH 7.0 germination never occurred, though the spores remained viable.

Like the vegetative cells, the germinating spore is sensitive to changes in pH. Most spores fail to germinate in an acid medium. It appears that a neutral pH, or one that is slightly alkaline is most favorable. The aerobic spore-formers are usually unable to germinate in media where the pH is less that 5.5, and the same thing appears to be true for some of the anaerobic organisms. Spores of Clostridium botulinum are unable to germinate if the pH is less than 4.8.

In a favorable medium, the time required for germination is relatively short. This was studied by B. R. Swann (3). He determined the time required to germinate by observing how long it would take the spore to form a vegetative cell that was just ready to divide. Studying Bacillus anthracis, he found that unless the spores were old or dessicated, the time required was about one hour and 30 to 40 minutes. If the spores were allowed to age for a long period of time before they were suspended in the growth medium, the time was moderately increased. He believed that this was due to the effects of dessication so that more time was required for the spore to imbibe a sufficient quantity of water to initiate the growth process. In these studies, Swann also observed the effect of aging and drying on the viability of the spores. In young spores that were from ten to twenty days old, the number that failed to germinate was between five and six percent. In older spores that had been allowed to dry for some time, the percentage increased considerably up to about 55%. It is not clear from his studies, however, that these spores are necessarily dead. It may be that his time of observation was not long enough, and that if he had used a longer period of observation, a larger percentage of the spores might have germinated.

One of the most interesting phenomena that have been observed in connection with the germination of spores is the phenomenon of dormancy. It has been a common observation by many bacteriologists that frequently spores may remain as spores for long periods of time in a medium which is presumably favorable for growth. Thus Burke, Sprague, and Barnes (4) found that although the majority of B. subtilis spores develop in four or five days, some lie dormant for as long as 90 days. McCoy and Hastings (5) found delayed germination is some of the spores which they had picked by single-cell technique. They picked out a hundred such spores and inocu-

lated each separately into what was presumed to be favorable media. Out of these hundred spores, some of Clostridium acetobutylicum, and Clostridium pasteurianum, they found that six germinated only after a relatively long period of incubation. Apparently the balance of them germinated readily. Amongst these six spores, one germinated in eleven days while another one germinated only after 222 days. The latter one was an old spore that had been isolated from a culture of corn mash approximately a year old. However, even some of the young spores showed delayed germination. Thus one which was isolated from a freshly grown culture required 21 days for germination.

Delayed germination is observed frequently by most food bacteriologists. Anyone who has incubated canned goods that have been heat-processed has had the experience of observing cans spoiling after remaining in the incubator for relatively long periods of time without any apparent sign of spoilage. On being opened, such cans reveal the presence of one or more types of spore-forming organisms. The explanation offered is that the spores have remained dormant during this long period of incubation.

Some bacteriologists regard dormancy as an inherent property of the spores. However, there is considerable evidence to indicate that this is not the case. It may rather be due to an unfavorable medium into which the spores have been inoculated. There is considerable evidence in the literature now that spores can be adversely affected by a number of different types of inhibiting agents.

Foster, Williams, and co-workers (6) reported that the fatty acids, particularly the unsaturated fatty acids, interfere with germination. These men have found that if unsaturated fatty acids are present in the medium in which the spores are inoculated, germination may be delayed indefinitely or at least for long periods of time. They have found, for example, that if a spore suspension is inoculated into a medium containing these unsaturated fatty acids, and if this medium is used for the counting of spores, the resulting counts are unreasonably low. If the same suspension is inoculated into media that do not contain these unsaturated fatty acids, the spores will germinate in a relatively short time, and then give much higher counts. In case the concentration of the unsaturated fatty acids is not excessive, the effect can be counteracted by adding starch to the medium, and for this reason they recommend adding starch to media that are to be used for spore counts. Foster believes that other substances, such as albumin, may also act as agents that counteract the effect of the unsaturated fatty acids. As a result of the extensive studies made by Foster, he concluded that the unsaturated fatty acids represent only one class of compounds that can act as inhibitors of germination. He believes many other compounds, yet unknown, can have the same effect, but that most of them can be counteracted by the addition of starch to the medium.

In some recent studies made on media used for determining spore counts, it was apparent that starch did not counteract all the inhibitors present. We found that if we added to the medium that was to be used for the counting of the spores a fair amount of media from an old culture containing a variety of organisms, both spore formers and non-spore formers, low counts were obtained even though starch was present. It would appear that in this case microorganisms had elaborated these inhibitors either

through synthesis or as a by-product of the breakdown of the substrates.

The above observations bring up some very interesting questions. If one inoculates a medium that contains some of these inhibitors with a large number of spores, it will be found that germination of some of the spores will take place in a relatively short time and that one will soon have an actively growing culture. However, if the medium is inoculated with a relatively small number of spores, germination may be delayed for a long period. This raises the question of why a given concentration of inhibitor will prevent a small number of spores from germinating while it will allow some of the spores in a large population to do so. One explanation would be that the sensitivity of these spores towards the inhibitor may vary from spore to spore and that if a person uses a large inoculum, there will be a few spores that are relatively insensitive and therefore those will be the ones that germinate. However, if one uses a small inoculum, then the probability is high that such spores will not be present, and consequently germination will be delayed.

Another interesting question is that, if the spores cannot germinate immediately, why should they be able to do so after standing a long time? Does this mean that the sensitivity of the spores for the inhibitor will decrease on standing, or does the concentration of the inhibitor diminish on standing? Both of these, of course, could happen.

There is another phenomenon which has been observed in connection with the germination of spores that is of considerable interest. In studies made on some of the thermophilic spore-formers, it has been reported that the spores fail to germinated, or show delayed germination unless they are given the pretreatment with heat prior to inoculation into a favorable medium (7). It is apparently the opinion of the investigators who did this work that this is a property of the spore. According to them, the heat treatment alters the spore in such a way that it germinates more readily. While this may be a valid explanation, it is by no means the only possible one. This again could be due to the presence of inhibitors. This is indicated in some work reported by Christian (8). He studied the germination of some spore-forming thermophilic organisms isolated from commercially sterilized milk, and found that if the spores were inoculated into milk after they had been given a heat treatment, germination invariably occurred in a relatively short time. If, however, he added a small amount of a growing culture containing only vegetative cells to the suspension of spores which had been heat treated, and which possessed the power to germinate quickly, a number of the spores were so affected that they lost the power to germinate. If the spore suspension was allowed to remain in contact with the vegetative culture for 24 hours, all of the spores lost the ability to germinate. He postulated, therefore, that the vegetative cells elaborated some substance which would act as an inhibitor, and that this substance was thermolabile. Therefore, he felt, the effect of heating is to destroy the inhibitor rather than to alter the spore. This may well be the correct explanation wherever this particular phenomenon has been observed.

Mr. Christian made another very interesting observation. He reported that some of the spores of the thermophilic organisms failed to germinate even though they had been given a pretreatment with heat. However, if he added to these spores a small quantity of a heat-killed culture of vegetative cells that had mutated so that they could not produce spores, the

addition induced the spores to germinate. This indicates a very complex situation in which we may have both inhibitors and stimulators. If vegetative cells can produce substances that inhibit germination, as well as substances that stimulate germination, it is not unreasonable to suppose that chemicals can also be produced by organisms which will stimulate the formation of spores. This would be, indeed, a very interesting problem for investigation.

Effront (9) reported that spores give off a proteolytic enzyme that can attack vegetative cells; therefore, in a suspension in which there are large numbers of spores, the enzymes may inactivate and destroy the remaining vegetative cells. He also reported that the greater the activity of the spores from the standpoint of the secretion of such proteolytic enzymes, the more difficult it was to get the spores to germinate. It is probable, therefore, that some of the by-products which are produced when these proteolytic enzymes act on protein may give rise to inhibitors. Thus the spores themselves may be capable of producing substances that will interfere with their own germination, and it is only when they have been removed from the medium in which they are suspended and diluted sufficiently to remove the effects of these inhibitors that the spores will be able to germinate. This suggests, therfore, that if one should study the germination of spores in a heavy suspension, as compared to a dilute suspension, one should find that the percentage of the spores that germinate will vary considerably.

From the foregoing, one could expect that when a few spores have germinated in a spore suspension, the vegetative cells which are formed may produce substances which will prevent the rest of the spores from germinating, and thus give the kind of results that we found in one of the experiments to which I referred earlier in this manuscript, namely, that the spore count seemed to drop during the first 24 hours, but after that, it increased and stayed at a fairly high level. Perhaps some of the spores that germinated first produced vegetative cells which in turn elaborated substances that prevented the balance of the spores from germinating. This may explain why some food materials which are heavily contaminated by spore-forming organisms may be difficult to sterilize, even though the food products are handled in such a way that the spores should normally germinate and produce vegetative cells which are easily killed. If the spores are very abundant, then those which do germinate may produce vegetative cells which will elaborate inhibitors that will keep the balance of the spores from germinating. Thus the spores will remain viable and dormant in a medium that one would normally consider to be favorable for germination.

I should like to come back to this question of why, if one introduces a large number of spores into a medium that contains some inhibitors, some of them will germinate, whereas if only a small number are introduced, germination will fail to take place. I am not entirely satisfied with the more obvious explanation, namely, that this is due to differences in susceptibility to inhibitors amongst the individual spores. I am inclined to believe that this same type of phenomenon would be observed if all of the spores were exactly alike. What can one then offer as a possible explanation? It is a will-known fact that many microorganisms are sensitive to peroxides, and particularly to hydro-peroxides. This is particularly true of the anaerobes. Let us suppose, therfore, that these hydro-peroxides can act as inhibitors for germination. Many of these peroxides are likely to be present in media, particularly if these media contain unsaturated fatty

acids, and also if the media are made from dehydrated media where the fatty acids present have had a chance to come in contact with atmospheric oxygen. Some of these hydro-peroxides may be fairly stable. The peroxides can be decomposed by various types of enzymes, and there may be a small amount of such enzyme activity in spores. If one introduces a large number of spores, there will be sufficient amounts of enzymes present so that the hydro-peroxides can be inactivated, and thus allow the spores to germinate. However, if a small number of spores are introduced, the peroxides may remain stable for relatively long periods of time. However, the peroxides them selves are unstalbe, and upon standing a long time, they will gradually disappear. Therefore, as time passes the medium may become more and more favorable for growth, until finally it reaches a point where the spores can germinate. If experiments are designed to test such an hypothesis, I am quite certain that the information gained would be useful and would lead to a better understanding of the process of germination.

Even though our information is rather meager, it does appear that microorganisms can elaborate substances which may either interfere with or which may stimulate germination. It certainly would be worthwhile to carry out further studies in this regard. It should be possible now to isolate and identify these inhibitors, and this would give us valuable information about the process of germination. Also, if we could find and isolate the compounds that stimulated germination, they might prove to be very useful.

It is hoped that these few remarks which I have made concerning the germination of spores will stimulate an interest, and not only provoke discussion in this meeting, but also stimulate fundamental research so that our basic knowledge may be considerably expanded.

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IX

WHAT IS STREAM POLLUTION?

M. D. Sanders
Swift & Company, Chicago

Stream pollution abatement is important to our welfare when it pre-
serves or restores the valuable assets of our surface waters. It is impor-
tant to our economics because of the tremendous potential cost to the pub-
lic and to industry of building and operating works for treating the sewage
and industrial wastes that are now discharged to surface waters.

Therefore, it seems advisable to try to balance the expected gains
in surface water resources against the cost of securing these gains, bear-
ing in mind that the innocuous disposal of sewage and wastes is one of
these resources. It naturally follows that the term, "stream pollution",
should depend on the effects of the waste products of our modern econ-
omy on the other uses of our streams.

Most of our great cities and industries grew up along our major ri-
vers, around the Great Lakes or on the sea-coast. The economical and in-
offensive disposal of sewage and industrial wastes into these waters was
undoubtedly one of the circumstances that made possible their growth.

These adjoining waters offered many other services, which, with ur-
ban and industrial growth, began to overlap, and finally to conflict in some
cases. For instance, intensive use of inland waters for public bathing, nav-
igation, or sewage and waste disposal began to interfere with their use as
a source of potable water. On the sea-coast shell fishing had to give way
in some places to harbor and waterway development, and sometimes to
sewage disposal.

The tremendous increase in population and industry along our rivers
and lakeshores has multiplied many-fold our use of their water resources.
This intensity of use was dramatically pointed out in the 1936 report of the
National Resources Commission, which said, "One quart of every gallon of
Ohio river water has at one time or another passed through a sewer." (1)

The problem of stream pollution derives its importance from its ef-
fect on the other valuable resources of our surface waters. Obviously, if
the other desirable and necessary uses are not affected, there is no prob-
lem of stream pollution.

A very clear statement of the place occupied by sewage disposal, in
the intelligent and conservative use of our surface waters, was published
in Public Health Reports (2) 30 years ago. The two paragraphs of this state-
ment read as follows:

"The disposal of sewage by dilution consists of its discharge into
natural bodies of water, in some cases with, and in others without, pre-
liminary treatment. In the former, it consists of a single and complete
method of disposal. In the latter, it may be regarded as a final step of a
more or less elaborate system of treatment, in which it is employed in

60

conjunction with one or more artificial processes. Wherever sewage or a sewage effluent is discharged into a natural water course, dilution constitutes an integral part of the disposal process.

"Dilution has for a long time been the most common measure of sewage disposal practiced in the United States. Formerly, it was regarded as a temporary and undesirable expedient, to be ultimately abandoned as soon as sewage purification became sufficiently developed. During recent years, however, the economic need for utilizing to the greatest possible extent the self-purification capacity of natural water courses has been apparent, with the result that the disposal of community wastes by natural dilution has become recognized as a legitimate and desirable process where carried out in such a manner as not to endanger the public health."

Nowadays, there are several other criteria, in addition to the effect on public health, that limit the disposal of untreated wastes into surface waters. There is also extensive data, resulting from the researches of public health scientists, which tell us the rate of destruction of pollutants in natural waters, and therefore, the rate of recovery of these waters to acceptable purity.

A summary of the general requirements of surface waters, with respect to their various uses, will serve to round out the problems of pollution.

First, with respect to public health, the raw water supples of purification plants must not be contaminated with the bacteria of sanitary sewage, or chemicals from industrial waste beyond the capacity of the water plant to remove them. But if there is no water supply taken from the stream in vulnerable range of the source of sewage or industrial waste, regulations should not impose the rigid conditions of waste treatment that a nearby water intake would entail.

Second, the fishing and recreational aspects should be considered, if the stream is in an area of low enough population density and has the other attributes of a recreational area. The organic load to the stream will have to be adjusted to permit the maintenance of minimum dissolved oxygen concentrations, and poisonous materials will have to be limited. Streams in densely populated and industrialized areas are usually excluded from the high dissolved oxygen requirements of fishing streams. The impracticability of maintaining balanced fish life or bathing beaches in such streams as the upper Illinois River system, the Hastings Pool in Minnesota and in congested coastal waters has been wisely recognized by most public health authorities.

Third, agricultural uses, such as irrigation of vegetable crops and the watering of livestock must be protected. The sanitary significance of sewage polluted waters in connection with dairy herds is well known, and bacterial quality must be of a high order in water for irrigation of crops that are eaten raw.

Fourth, industrial water supplies for other than food uses must be protected from corrosive chemicals and usually are desired at the lowest possible temperature.

Fifth, navigation and water power are usually modest in their requirements. Deposits that block channels and fill reservoirs, and corrosive elements are usually the only conditions objected to by users of these resources.

A great deal has been learned about the safe limits of bacterial count

in surface waters that are to be processed for potable uses. Extensive studies have also been made of the bacterial death rate in natural waters. Since public water supplies impose the most exacting requirements of all uses on surface streams, it seems advisable to discuss this subject in more detail.

Bacterial limits in raw water for public water purification plants were the subject of a recent study by H. W. Streeter (3). He ascertained the efficiency of bacterial removal of a large number of municipal plants on inland rivers and the Great Lakes. His conclusions were that a well operated, modern water filtration plant can be expected to consistently produce a safe water if the E. Coli concentration in the raw water never exceeds 5,000 per 100 ml. He found that, where chlorination is the only treatment given, as in certain Great Lakes cities, 50 E. Coli per 100 ml. of raw water could not be exceeded to assure a safe drinking water at all times. These bacterial concentrations have come to be recognized in general as the upper limits permissible at the raw water intakes of public water purification plants.

These limits tell us what the condition must be at the intake of the water plant but they do not tell us what bacterial limits must be maintained 10 miles or 50 miles upstream.

The rate of bacterial destruction in natural waters has been investigated during many river surveys by public agencies, notably the U. S. Public Health Service.

The accompanying .curve, (4) taken from Phelps' "Stream Sanitation" summarizes Ohio River studies in the 1920's. It relates the death rate of E. Coli to time elapsed below their point of highest concentration below the sewers of Cincinnati. It will be seen that in 2 days, 90% of the bacteria have succumbed to their unfavorable environment and at the end of 10 days, in warm weather, only 1/10 of 1% of the original numbers are still alive. The second figure is taken from the report of a survey of the Upper Mississippi River by the U. S. Public Health Service (6). This compares bacterial death rates in the Upper Mississippi with rates determined for the upper and lower Illinois River, and the Ohio River between Cincinnati and Louisville. Allowing for great differences in initial bacterial population, and in varying additions of bacteria along the courses of the rivers surveys, the curves agree very well in their general trends, except for the Mississippi plate counts beyond 100 hours. These variations have been adequately explained (7) but are not particularly pertinent to this discussion.

It is apparent from these data that bacterial destruction in natural waters is extremely rapid. Th author recalls visiting a public water purification plant on the heavily polluted Hoogly River, near Calcutta, India. He was told that counts of 5,000,000 per 100 ml. were common in the raw river water, pumped to the open impounding reservoir. Yet in the 30 day's storage that ensued, counts dropped consistently below 1,000, providing a satisfactory raw water for the purification plant.

It is therefore apparent that, given a reasonable time which will vary according to the magnitude of initial bacterial concentration and with other factors such as temperature, turbidity, and pH, bacterial concentrations can reach acceptable limits for any purpose in natural waters. If the time for natural improvement is not available, then the downstream water plants must be safeguarded by sewage treatment upstream, sufficient to reduce the bacterial load to safe limits at the water plant.
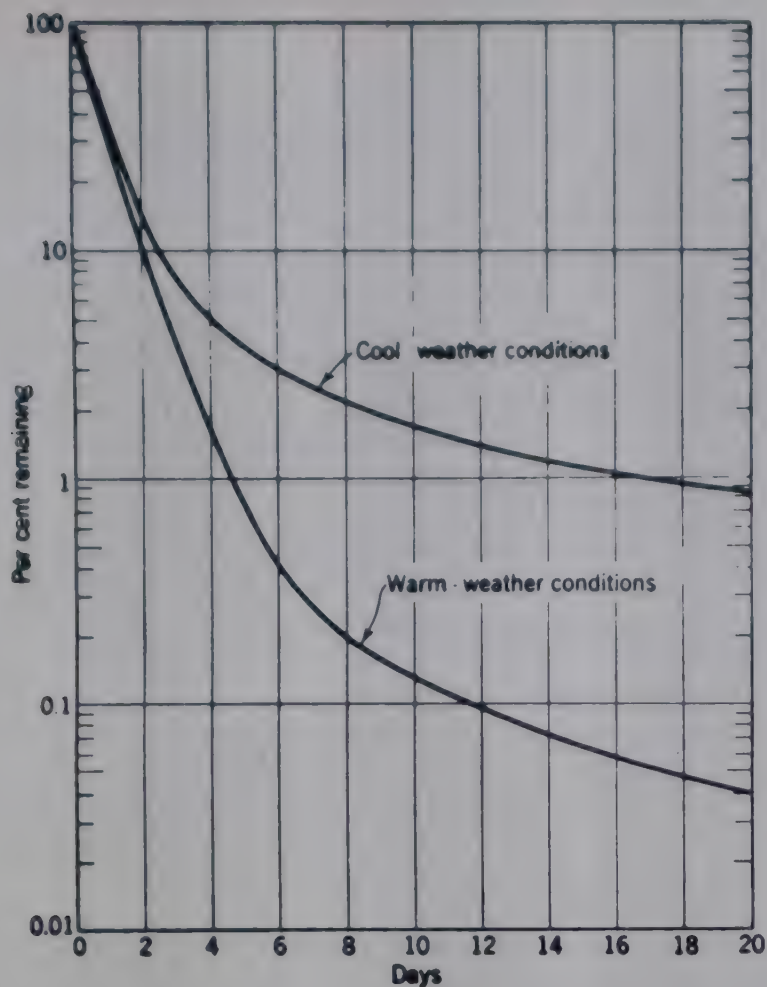
Figure 18. Death rate curves of E. coli in the Ohio River. Reproduced by permission from STREAM SANITATION by E. B. Phelps, published by John Wiley & Sons, Inc., 1944.
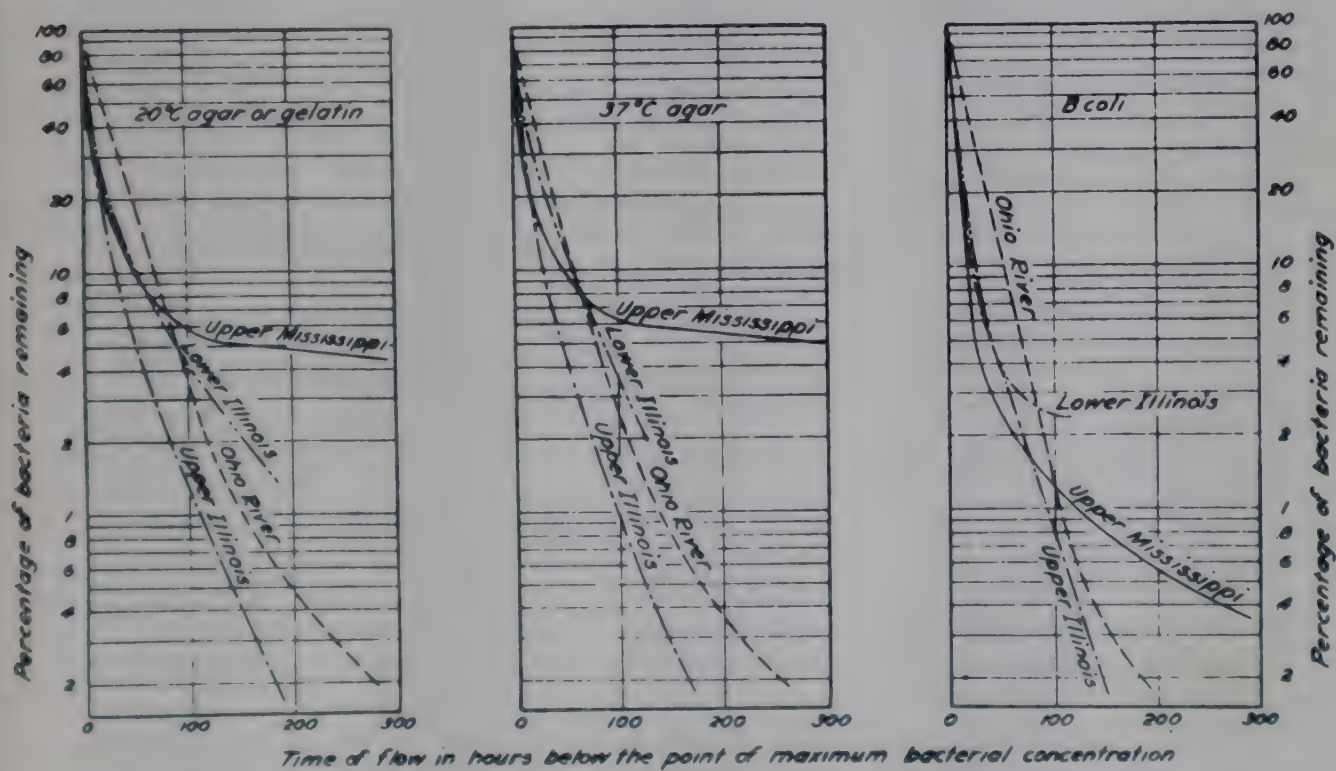

Figure 10. Comparison of summer rates of bacterial purification in the Ohio, Illinois and upper Mississippi Rivers in relation to time of flow from the zone of maximum pollution. From Public Health Bulletin #203, p. 105.

In other words, if upstream sewage disposal interferes with necessary use of the water downstream, then the logical solution is to adopt a method of disposal that will not interfere with the downstream use.

Two other features of the pollution question that deserve further discussion are maintenance of fish life and prevention of nuisance. They impose rather definite, and similar, conditions on surface waters that are used for the final disposal of wastes. Industrial waste disposal is far more often affected by the requirements of these factors than by the requirements of public water supplies.

There is a large background of research on the two fundamental factors in this phase of surface water control, which revolve around dissolved oxygen content and biochemical oxygen demand.

Biochemical oxygen demand (BOD) is the measure of the amount of dissolved oxygen consumed during bacterial destruction of organic matter in water. Nuisances, in the form of odors and discoloration, develop when dissolved oxygen is consumed at a greater rate than it can be supplied by incoming aerated water, and by solution from the atmosphere. In the absence of oxygen, certain bacteria continue to work, but their oxygen is derived anaerobically from proteinaceous materials, sulphates, and other oxygen containing compounds. These materials sometimes yield foul odors, such as hydrogen sulphide, when robbed of their oxygen by bacteria.

The course of the BOD reaction is illustrated in the accompanying figure, taken from Public Health Bulletin #173. (8) Although three temperatures of incubation are shown here, 20º C. is the temperature usually chosen for laboratory determinations. Definite mathematical relationships exist between different temperatures, so oxygen depletion can be estimated at other temperatures from the 20º result. Depletion of dissolved oxygen in water due to bacterial activity has been found to follow a definite course. Thus, if we take the 20-day BOD value as 100, on the 20º curve, the one-day value will be 20.5 and the 5-day value will be 68.5. Expressing the rate of demand the way the physicists describe the disintegration rates of radioactive material, the BOD has a "half-life" of 3 days.

The effect of decomposing organic material on the oxygen content of a stream is illustrated in the next figure, which is from Phelps' "Stream Sanitation" (5). It is apparent that the "Oxygen Sag Curve" is the result of combining the "Reaeration" and "Deoxygenation" curves. It gives us the net oxygen deficit when oxygen demand is compensated by reaeration.

The formulae on which these two figures are based permit us to predict the effect of organic wastes on surface waters with respect to nuisance production, since the dissolved oxygen in the water at any later time can be calculated. If fish life is important in the stream, the effect of the waste on that factor can be assessed in the same manner.

With this background, we should be able to develop a workable definition of pollution. It must be general, yet impose all of the restrictions that have been discussed, but only when these restrictions are necessary to protect other important uses of the stream. I believe the following definition might fit this complex set of requirements:

"Pollution occurs when materials are added to surface water that prevents the necessary and normal use of the water or its land environment."
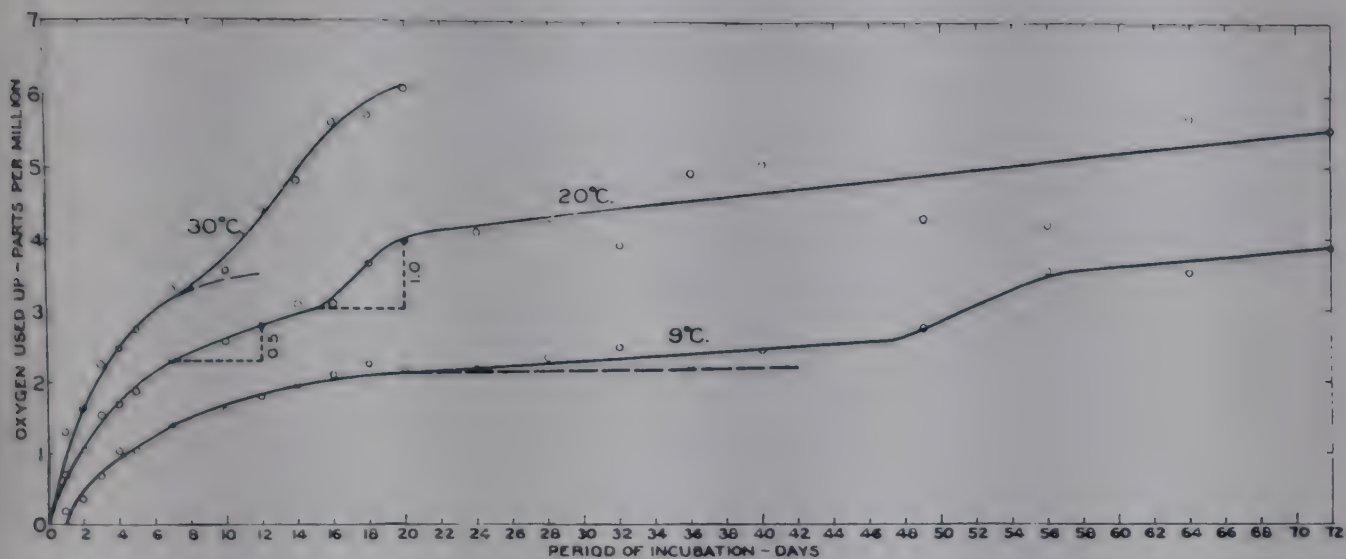
Figure 1. The general course of the deoxygenation curve. From Public Health
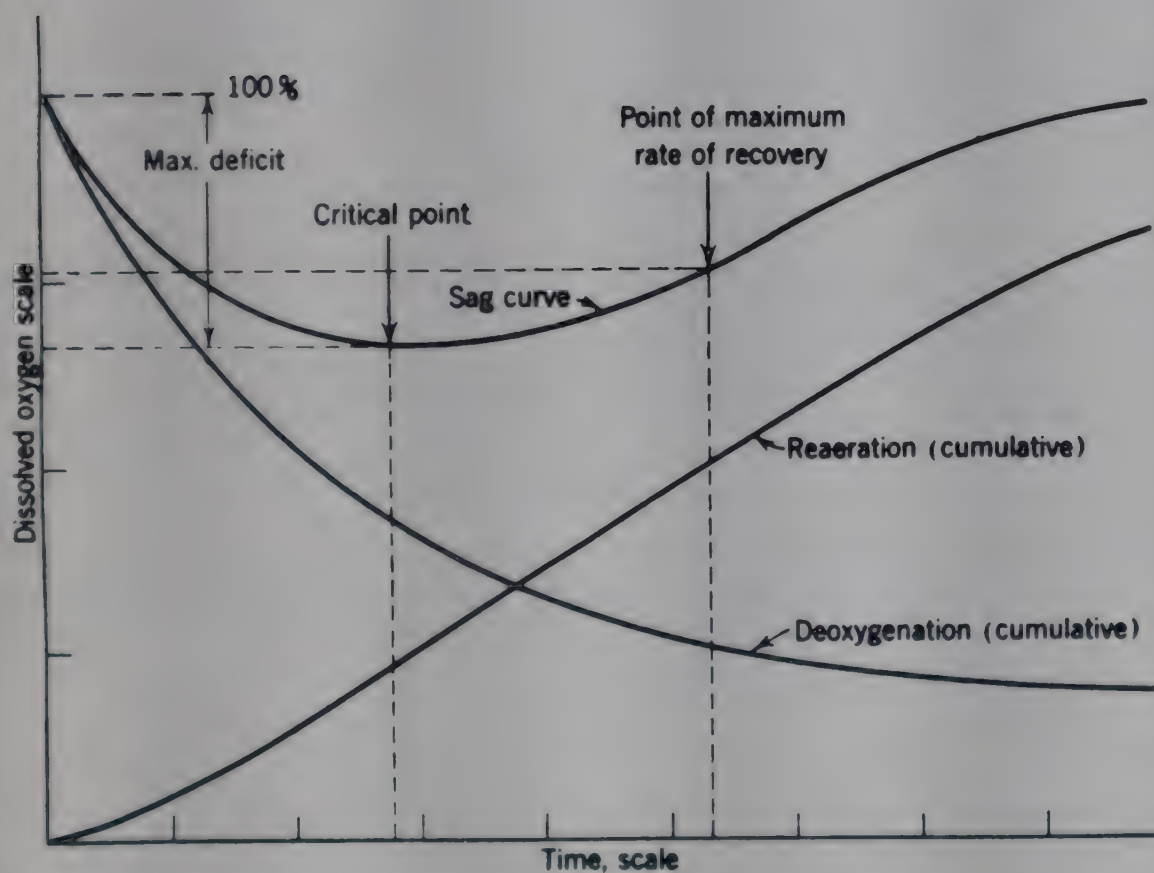Bulletin #173, p. 132.

Figure 14. Deoxygenation, reaeration, and sag curve. Reproduced by per-
mission from STREAM SANITATION by E. B. Phelps, published
by John Wiley & Sons, Inc., 1944.

The National Resources Committee reported (9) in 1939 that the estimated cost of necessary new construction for industrial waste treatment as of 1937 was $900,000,000 and for new municipal sewage plant construction, $1,000,000,000. If these figures were adjusted to present day costs, they would be about $1,800,000,000 for industry and $2,000,000,000 for municipalities. From the same source we learn that the added annual cost of such facilities might approximate a half billion dollars annually, with 9/10 of the cost falling on industry.

It is further reported that $30,000,000 was needed in 1937 to provide waste disposal facilities for the meat packing industry. If we adjust this figure to today's costs, we find the amount would be $60,000,000, with a $15,000,000 annual operating and maintenance bill, further interpolating the reference quoted. It is probably impossible to estimate what the meat industry is currently spending on industrial waste treatment. However, the present cost of production of the almost 20 billion pounds of dressed meat annually in the United States would be increased by 7-1/2 cents per hundred weight by an added expenditure of $15,000,000 annually for waste treatment by the industry.

Such figures should make us realize the importance of the definition of stream pollution, on which requirements for municipal and industrial waste treatment plants will be based. We neither want to waste our surface water resources through pollution, nor, by over-shooting pollution abatement measures, do we want to waste our resources of capital and materials by building and treating beyond the practical needs of our surface waters. Therefore, stream pollution, in terms of allowable tons of BOD per day, or allowable concentrations of E. Coli, must be redefined for almost every mile of every stream if we are to attain a full and balanced utilization of the resources of our surface waters.

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X

THE IDENTITY OF STAPHYLOCOCCI
ASSOCIATED WITH FOOD POISONING

James B. Evans
American Meat Institute Foundation

The bacteriological investigation of foods suspected of being involved in a food poisoning outbreak is seriously handicapped by the lack of dependable, yet simple, laboratory tests that will identify enterotoxigenic staphylococci. The basic reason behind this situation has been a rather widespread opinion that staphylococci of widely varying physiological characteristics are capable of causing food-poisoning. This opinion has been brought about primarily as the result of the rather common practice of designating as a known food poisoner any Staphylococcus isolated from a food item accused of causing food poisoning. This is particularly true if the organism happens to be orange pigmented, or if it hydrolyzes gelatin. A physiological study of a collection of such organisms will invariably show them to be a rather heterogeneous group. The reasons for this are fairly obvious when we realize that saprophytic staphylococci are commonly present in the air and on the skin, and that they can grow under widely diverse conditions. The presence of small numbers of these organisms must be expected on almost any non-acid food which is exposed to the air or to human hands. The average individual, when made ill, has a tendency to blame certain types of food without considering other foods which are just as capable of causing the trouble. The suspected food is usually grossly mishandled before it reaches a laboratory, and subsequent examination is almost certain to show the presence of staphylococci, and frequently in large numbers.

However, when the label "enterotoxin positive" or "known food poisoner" is reserved for strains which have been grown in pure culture and tested by controlled feeding tests on human volunteers or monkeys, a different picture emerges.

A study has been completed in our laboratory in which the physiological characteristics of a large collection of staphylococci have been compared with their ability to produce enterotoxin experimentally. Enterotoxin production was determined by growing the cultures under 20 per cent CO_2 on semi-solid veal infusion agar, and feeding 50 ml. portions of filtrate by stomach tube to rhesus monkeys. Some of these feeding tests were conducted in our laboratory; the others were conducted by Dr. Dack, Dr. Surgalla, and their associates here at the University of Chicago. The results of these studies indicate that all food-poisoning staphylococci are members of a well-defined and nomogeneous group, that may be designated as the coagulase-positive group (that is, they induce clotting of citrated rabbit blood plasma).

As shown in Table I, all enterotoxic strains were coagulase-positive; there was no evidence of enterotoxin production by any of the coagulase-

negative strains tested. The 25 coagulase-negative cultures were mostly isolated from foods suspected of causing food poisoning, and were fed to

Table I

	Enterotoxin positive	Enterotoxin negative
Coagulase positive	32	12
Coagulase negative	0	25

a total of 61 monkeys without producing any symptoms of food poisoning. In contrast to these results, 5 coagulase-positive strains isolated three years ago from wholesome frozen foods were tested, and four of these five strains were found to be positive for enterotoxin production. In addition, of six coagulase-positive strains recently isolated from such clinical sources as nasopharyngitis, mastoid infection, and a boil, four gave a positive test for enterotoxin.

The coagulase-positive strains comprise an extremely homogeneous physiological group, and there is no apparent difference between those which produced enterotoxin and those which gave no toxic reaction in these tests. The single physiological test which correlated best with the coagulase test was the ability to ferment mannitol under anaerobic conditions. Among 114 cultures there was a 98 percent correlation. Reports in the literature also indicate a high correlation between the coagulase test and the production of hyaluronidase, as well as the production of alpha lysins.

Based upon the results obtained in our laboratory and the published results of other investigators, we would like to make a few statements regarding the laboratory examination of foods accused of having caused food poisoning:

First, it is impossible to state positively that a certain food item did not cause food poisoning unless the food in question is fed to several volunteers with no ill effects. This is a rather impractical procedure, particularly in view of the condition of most samples by the time they reach the laboratory. The biggest reason for this impossibility is the fact that the enterotoxin, once it is produced, is much more heat resistant than the organismsm s which produced it.

Second, while the presence of large numbers of coagulase-negative staphylococci on a food item may be indicative of gross mishandling at some point, there is no evidence that consuming this food would have any noticeable effect upon the well-being of the consumer. As pointed out previously, the finding of small numbers of saprophytic staphylococci is to be expected in non-acid foods exposed to the air or to human hands.

Third, any food that contains large numbers of coagulase-positive staphylococci should be considered potentially dangerous. Furthermore, any food containing small numbers of coagulase-positive staphylococci might become dangerous if inadequately refrigerated.

Fourth, the classical tests such as gelatin hydrolysis, orange pigmentation, and mannitol fermentation are of little or no differential value in separating the food poisoning staphylococci from the saprophytic

68

types. Reliance on such tests serves only to confuse the examination of a food for enterotoxic staphylococci. We will give three examples:

In one case we received a ham which was accused of having caused the sickness of approximately 50 out of 100 guests at a wedding reception. As is usually the case, we were unable to learn much about other foods or beverages consumed at the reception. By the time we received the ham (un-refrigerated) the surface had approximately 2 billion bacteria per gram. The majority proved to be orange-pigmented staphylococci which gave a positive Stone's gelatin test and fermented mannitol in the presence of 7.5% salt. We feel certain that most laboratories would consider these organisms to be food poisoners on the basis of this evidence. However, the cultures studied from this ham were coagulase-negative and failed to ferment mannitol anaerobically. Two of these cultures which we considered typical of the majority present on the ham were studied further, and were tested for enterotoxin production. One culture was fed to 5 monkeys and the other to 3 monkeys with completely negative results.

In another case we received two samples of dried beef which were accused of making members of two different families extremely ill. One of these samples contained 180 million and the other 2 million bacteria per gram. In both cases these were staphylococci. Nine cultures selected at random were all coagulase-negative, and 2 were selected for further study. One was white, fermented mannitol, and hydrolyzed gelatin; the other was cream colored, fermented mannitol, and did not hydrolyze gelatin. The white strain was fed to 5 monkeys and the other strain to 3 monkeys, all with negative results.

In a third case, we received from another laboratory a white strain of Staphylococcus isolated from a ham accused of causing food poisoning. This culture gave a negative Stone's gelatin test, but was coagulase-positive and fermented mannitol both aerobically and anaerobically. This culture was fed to 2 monkeys. One of the monkeys manged to spit out the entire feeding as the stomach tube was withdrawn. The monkey which retained the feeding became extremely sick, vomiting and retching repeatedly, begining about 80 minutes after the feeding.

Another interesting story grew out of this last case. The feeding which was spit out by the first monkey soaked the knees of the trousers worn by one of the attendants. Approximately 30 minutes elapsed before this worker removed the trousers and washed with soap and water. Within 24 hours, 7 or 8 small local hair follicle infections developed around one knee. Later 4 or 5 more of these pyogenic lesions appeared on the other leg. Material was taken aseptically from 2 of these lesions and white strains of coagulase-positive staphylococci were isolated. No such organisms were found in scrapings from a non-infected area. Since the recovered micro-organisms proved to be identical to the strain used in the feeding test, we feel certain that this represents a case of laboratory infection by a food poisoning Staphylococcus. One of these re-isolated strains was fed to two monkeys and typical symptoms of enterotoxin poisoning were produced in both animals. This laboratory accident is not too unexpected, since our data show that pathogenic and enterotoxigenic staphylococci are identical with respect to every physiological test employed. The physiological reactions of these organisms have been omitted from this present discussion, but are included in a paper that is now in press in the Journal of Bacteriology.

XI

ENZYMES AND THEIR IMPORTANCE IN THE
MEAT PACKING INDUSTRY

D. M. Doty
American Meat Institute Foundation

Enzymes are naturally-occurring catalysts that initiate and control many, if not most, of the reactions which result in the physiological processes of all living tissue. They are the "traffic policemen" that direct the thousands of changes taking place during the growth, development, and sen escence of any living cell. Also, they continue to act after the death of the cell or tissue, but often in somewhat different manner than in living organ isms.

For this reason it appears logical, even essential, that any person who investigates or handles biological tissue, living or dead, should have some understanding of enzymes and the role they have in many of the read ily detectable changes occurring in biological materials. I shall attempt today to present very briefly some of the basic concepts of enzyme chemistry, to outline the factors that may influence enzymes and enzyme-catalyzed reactions, and to discuss some of the enzymes that are of particula interest to the meat packing industry.

What are Enzymes?

Before attempting to characterize enzymes completely, we should perhaps describe the type of reactions which require enzyme catalysis. Chemical reactions may be conveniently divided into two classes:

1. Those which are practically instaneous, for example

$$\overset{+}{Na}\overset{-}{Cl} + \overset{+}{Ag}\overset{-}{NO_3} \longrightarrow \underline{AgCl} + \overset{+}{Na}\overset{-}{NO_3}$$

$$\overset{+}{Na}\overset{-}{OH} + \overset{+}{H}\overset{-}{Cl} \longrightarrow H_2O + \overset{+}{Na}\overset{-}{Cl}$$

2. Those which require a measurable time to reach equilibrium, for example

$$RCO_2R' + H_2O \overset{OH^-}{\underset{}{\rightleftharpoons}} RCO_2H + R'OH$$
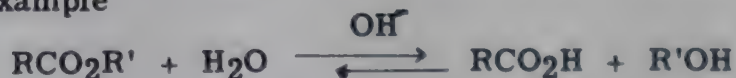
It is the second type of reaction that requires the presence of a catalyst for rapid and efficient completion. In living organisms there are many such reactions taking place; e.g. hydrolysis of starch to sugar, oxidation of sugar to carbon dioxide and water, synthesis and degradation of proteins. These reactions require drastic conditions in the laboratory but proceed smoothly at low temperatures in biological tissue. The catalysts responsible for these biological reactions are called "enzymes." They may be defined as "definite material catalysts, organic in nature, with specific powers of reaction, formed by the living cell but independent of

the presence of the latter in their operation." In the moist state they are heat-labile. All enzymes that have been purified and characterized up to this time are proteins or contain specific protein fractions. Therefore enzyme chemistry really becomes a branch of the larger field of protein chemistry.

Factors Affecting Enzyme — Catalyzed Reactions

Since enzymes are specific, heat-labile proteins, many factors influence their activity. In fact, anything which changes the physical or chemical nature of proteins would logically affect an enzyme and consequently the reaction catalyzed by it. Some of the more important factors are outlined below:

Reaction of the Medium (pH). The hydrogen ion concentration of the medium may influence enzyme action in several ways — (1) by influencing the way in which the enzyme protein dissociates; (2) by altering the physical state or colloidal nature of the enzyme protein, thus changing the area of the enzyme surface and indirectly its activity; (3) by affecting the substrate; (4) by altering certain specific inhibitors or activators present in the medium. The best (or optimum) pH for an enzyme-catalyzed reaction depends then on a number of factors including the nature and concentration of substrate, type of buffer, purity of the enzyme preparation, and length of time over which the enzyme acts. Figure 1 shows the influence of pH and type of buffer on the activity of urease.

Specific Activators, Kinases, Co-Enzymes, Inhibitors. Many enzymes are secreted as inactive pro-enzymes or "zymogens" which become enzymatically active only in the presence of specific activators. For example, "pepsinogen," the precursor of pepsin in the stomach is activated by H-ions. Arginase, the enzyme catalyzing the degradation of arginine to ornithin and urea, is activated by Mn^{++}. If the special activator is organic and heat-labile, it is called a "kinase." For example, trypsinogen is activated by "enterokinase" to form active trypsin. Some enzyme proteins must be combined with specific non-protein prosthetic groups before they exhibit activity. These special prosthetic groups are termed "co-enzymes." Many of these co-enzymes are vitamins; e.g. thiamine (vitamin B_1) diphosphate is co-carboxylase, the co-enzyme for carboxylase which is responsible for the decarboxylation of pyruvic acid in biological oxidation. Many investigators feel that all vitamins exert their biological activity by acting as co-enzymes for essential, specific enzyme systems in living cells. Just as enzyme proteins may be activated by activators, kinases or co-enzymes, so may they be inhibited or paralyzed by other compounds. Heavy metal ions, such as Ag^+, Cu^{++}, or Hg^{++}, inhibit some enzymes (e.g. invertase) and completely inactivate others (e.g. urease). In addition there are naturally-occurring organic inhibitors which may combine with an enzymatically-active protein and prevent its action on the appropriate substrate. For example, the trypsin inhibitor present in soybean prevents trypsin of the small intestine from digesting proteins at a normal rate.

Temperature. Since enzymes are proteins they are subject to heat denaturation and lose their activity at whatever temperature the essential physical structure or chemical groupings are modified. Thus the so-called "optimum" temperature for an enzyme-catalyzed reaction is that temperature at which increased reaction velocity due to heat most exceeds the des-

71
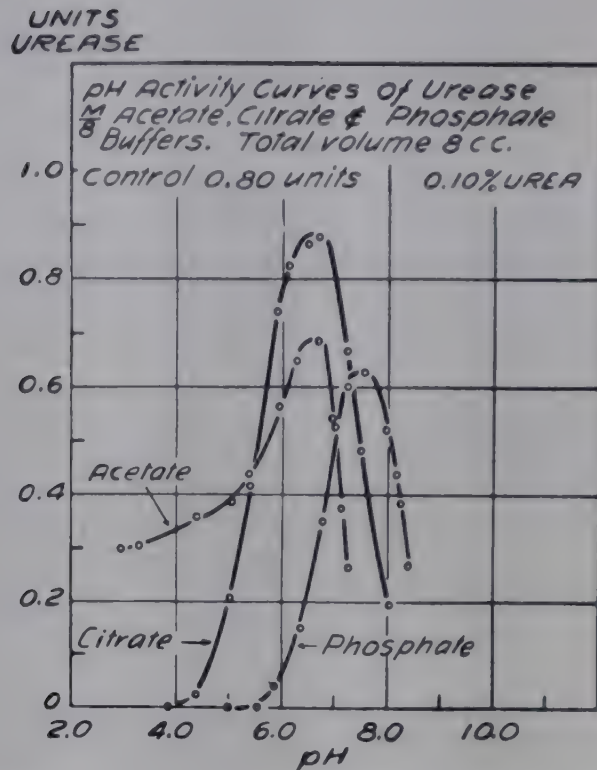
Figure 1. The influence of pH and type of buffer upon the activity of urease. (Howell and Summner, J. Biol. Chem. 104:619, 1934).
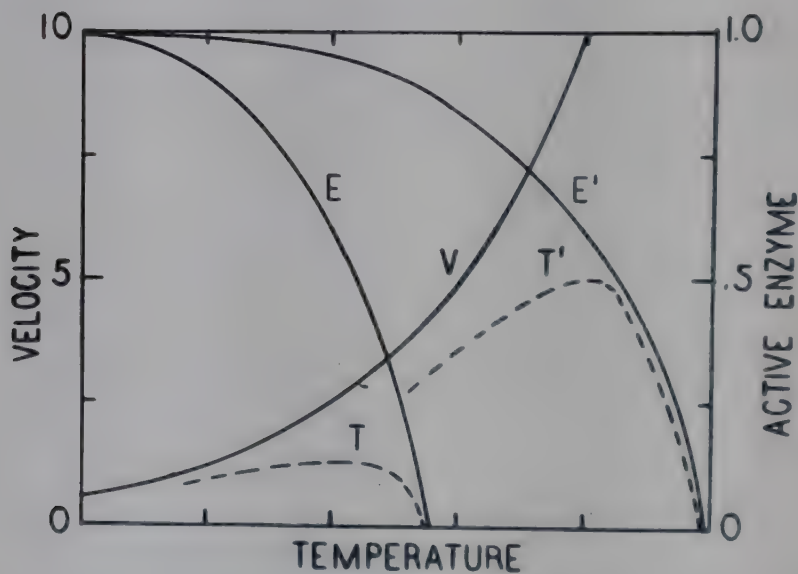
Figure 2. The influence of temperature on a reaction whose velocity increases with temperature (curve V), with active enzyme concentrations E and E' for long and short period reaction times respectively. The products EV and E'V, plotted as T and T' respectively, represent the overall velocity of the reaction for long and short periods.

truction of the enzyme by heat. Actually there is no certain optimum tem-
perature for a given enzyme reaction since the temperature for apparent
maximum activity varies with time of action, substrate concentration,
purity of the enzymes, and other factors. Figure 2 shows how the apparent
"optimum" temperature varies with time of action.

Enzymes and Substrate Concentrations. The specificity of enzymes
suggests that a union of rather intimate nature takes place between en-
zyme and substrate, which leads directly to the general theory that the
velocity of an enzyme-catalyzed reaction is proportional to the concen-
tration of the enzyme-substrate complex which is in turn proportional to
the concentrations of enzyme and substrate. This theory was first pro-
posed by Michaelis and Menten (Biochem. Z. 49:333, 1913) and has been
well-substantiated by experimental research since then. This enzyme-
substrate complex formation and decomposition may be represented as
follows:

ENZYME + SUBSTRATE $\rightleftharpoons$ COMPLEX $\rightleftharpoons$ ENZYME + END PRODUCTS

It can be shown mathematically and proved experimentally that the
velocity of an enzyme-catalyzed reaction is directly proportional to en-
zyme concentration when the enzyme-subtrate union is firm and/or the
substrate concentration is comparatively high. When the complex union
is loose and/or the substrate concentration is comparatively low, the
velocity is dependent on both enzyme and substrate concentration. On
this basis the general course of a enzyme-catalyzed reation can be plot-
ted as shown in Figure 3.
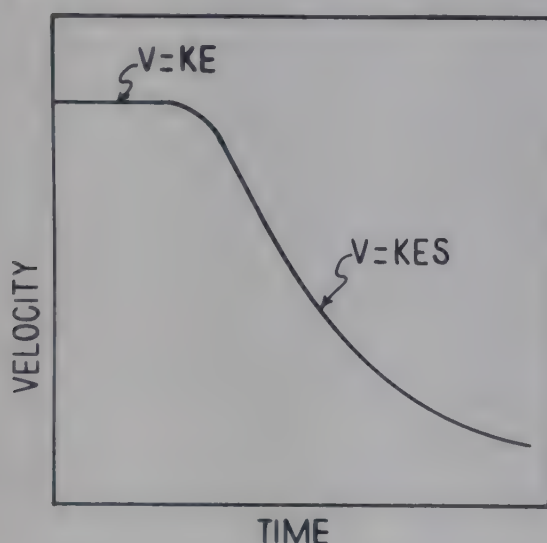
Figure 3. The velocity of an enzyme-catalyzed reaction as influenced by time.
With high substrate concentrations velocity is directly proportional to enzyme
concentration (V = KE); as the substrate is exhausted, the reaction rate is de-
pendent on both enzyme and substrate concentration (V = KES).

Other Factors. Radiation, particularly in the ultraviolet, may inac-
tivate enzymes (e.g. urease). High pressure and mechanical grinding have
been found to reduce enzyme activity in some cases.

Enzyme Specificity and Classes of Enzymes

In general it may be stated that a given enzyme will catalyze only a
single type of reaction with a single compound or type of compound. It may

catalyze the reaction in either direction, depending on the concentration of reactants, temperature, and other conditions. This specificity is based on the fact that the enzyme combines with the substrate to form a complex, as previously described, as the first step in the reaction. In order for the complex to form, the enzyme must have reactive groups in the proper steric configuration to combine with the reactive groups of the substrate molecule. In other words, the enzyme "key" must fit the substrate "lock." It is not surprising then that there are thousands of enzymes which catalyze the thousands of reactions which occur in biological tissue.

Enzymes are usually classified on the basis of the type of reaction catalyzed and the type of compound attacked. Thus we have a large group of enzymes known as "Hydrolyzing Enzymes" because they catalyze hydrolysis reactions and another large group known as "Oxidizing Enzymes" because they catalyze oxidation reactions. Enzymes which break (or form) carbon chains are called "Desmolizing Enzymes."

Each of these three main groups of enzymes is subdivided into smaller groups. These subgroups are usually established on the basis of the type of substrate attacked by the enzyme, or on the basis of some other special characteristic of the enzyme. For example, the main groups of the hydrolyzing enzymes are the esterases, carbohydrases, and proteolytic anzymes which hydrolyze esters, carboydrates and proteins respectively.

Enzymes Important in the Meat Packing Industry

There are three main ways in which enzymes are important in the meat packing industry: (1) as a by-product; (2) as a tool or reagent in the plant or laboratory; (3) as an agent that may greatly affect the characteristics of the main products produced by the industry. I shall discuss the first two very briefly and the last in somewhat more detail.

Enzymes as By-Products. Since enzymes are produced by living cells and remain active in the tissue after death of the organism, it is possible to prepare very active concentrates of many enzymes from certain specific tissues. In fact, many crystalline enzymes have been prepared. In the packing house there is a tremendous amount of by-product material that is an excellent source of certain enzymes. Some companies have taken advantage of this and produce enzymes for use in the pharmaceutical industry, laboratory supply, and other trades and industries that use enzyme preparations. Some of the enzymes prepared by the meat packing industry are rennin from calves stomachs, catalase from liver or erythrocytes, pepsin (or pepsinogen) from gastric mucosa of hogs, trypsin and ribonuclease from cattle pancreas, and phosphatase from intestinal tissue. Just a word of caution should be injected here. No company should attempt to purify and sell enzyme concentrates unless they have a good, experienced staff of chemists, biologists and development engineers to work out and control the processing.

Enzymes as Plant or Laboratory Reagents. In chemical research and control laboratories, enzymes are often used to catalyze specific reactions necessary for qualitative identification or quantitative determination of compounds. Urease, which catalyzes the hydrolysis of urea to water and ammonia, is used in the determination of urea; invertase, which catalyzes the hydrolysis of cane sugar to reducing sugars, is used in cane sugar (sucrose) determination; amylases, which split starch to sugars, are used in the determination of starch; various proteolytic enzymes are used to hydrolyze proteins in chemical and nutritional investigations on proteins.

One of the more important applications of enzymes in industrial process-
ing is the use of enzymes in the bating of hides in leather manufacture.
In bating the insoluble hair and epidermal residues are digested by pro-
teolytic enzymes. The soluble hydrolysis products may then be washed out,
leaving a firm, "grainy" texture.

Enzyme Action in the Preservation and Processing of Meat and Meat
Products. Earlier in this discussion it was pointed out that enzymes are
present in all living tissue and continue to exert activity after death of the
organism. Therefore, in the preservation and processing of meat the in-
fluence of enzymes must be recognized and evaluated because some of the
changes catalyzed by enzymes are desirable while others are undesirable.
Even some of the reactions that are desirable, if limited, may be very un-
desirable if allowed to continue unchecked for long periods. Perhaps the
most logical way to discuss these enzymes and reactions catalyzed by them
is by types of compounds affected.

Enzymes acting on fats. Two types of changes that occur in fats are
catalyzed by enzymes: (1) hydrolysis and (2) oxidation. You will recall
that fats are glycerol esters of fatty acids and may be hydrolyzed to gly-
cerol and fatty acids. This hydrolysis may take place at relatively low
temperatures under the influence of lipase normally present in fatty tis-
sue. Thus, if unheated fats or fatty tissue are held for long periods, even
at refrigerator temperatures, some of the fat may be hydrolyzed with the
consequent development of off-flavor and odor due to the presence of free
fatty acids. Under normal operations in a meat packing house, this change
is slow but may be a very critical factor in the production of high quality
products. If fats are held for appreciable periods of time before rendering,
free fatty acids appear as the result of lipase activity (Figure 4). These
fatty acids are not removed during rendering (unless the product is deod-
orized or alkali-refined) and the final product has a low smoke point. A
much more important change is the oxidation of fats by lipoxidase. This
enzyme catalyzes the addition of molecule of oxygen to certain double bonds
in unsaturated fatty acids, such as oleic, linoleic and linolenic, to form a
peroxide. The peroxidation of unsaturated fatty acids is the first step in
the development of rancidity and apparently follows the same general path-
way in the presence of lipoxidase as in its absence. It is of course much
more rapid in the presence of lipoxidase. Therefore, the oxidation of un-
heated animal fats may be quite rapid due to the presence of lipoxidase.
Unfortuantely, not very much is known about lipoxidase in animal tissues.

Carcasses Held	Average Free Fatty Acid		
	Due to Rendering	Due to Holding	Total
18-24 hours	////////	XXXXX	0.35%
96-120 hours	\\\\\\\	XXXXXXXXXXX	0.62%

Figure 4. The influence of carcass holding time on the free fatty acids of
lard rendered from the carcasses. (American Meat Institute Bulletin "Ma-
king Better Lard" 1944, p.5.)

75

Most investigators think that the lipoxidase activity is due to hemoglobin, myoglobin, or some other iron-containing (hemin) system, but none of the animal unsaturated fat oxidase systems have been well characterized or purified. Certainly additional investigations on animal lipoxidases would be very valuable to the meat packing industry because these enzymes undoutedly accelerate the development of rancidity in unheated fats and fatty tissue. For example, the development of rancidity in pork products such as bacon or hams might be slowed down if we knew how to inactivate the lipoxidase present. In processed animal fats, the lipoxidase has been inactivated by heat and autoxidation can be postponed by the use of a suitable antioxidant such as AMIF-72 (Tenox II), but such materials cannot be readily incorporated in intact fatty tissue.

Enzymes acting on proteins. Proteolytic enzymes, which catalyze the hydrolysis of proteins, are extremely important to the meat packing industry because practically all animal tissue is proteinaceous and therefore subject to hydrolysis by proteolytic enzymes.

We have considerable information about the proteolytic enzymes which are active in the protein digestion processes of the animal body. Pepsin in the stomach attacks native proteins and breaks them down to peptides. This enzyme is an "endo-peptidase" — that is, it catalyzes the hydrolysis of an interior peptide bond of the protein molecule. The presence of tyrosine or phenylalanine in the protein molecule adjacent to the peptide bond to be split is required for the action of pepsin.

Trypsin, which is secreted by the pancreas as inactive trypsinogen and is activated by enterokinase in the small intestine, is another "endo-peptidase" which acts on native proteins and primary protein degradation products. The presence of lysine or arginine in the protein molecule adjacent to the peptide bond to be split is required for trypsin action.

Chymotrypsin, another "endo-peptidase" active in the small intestine, acts similarly to trypsin but requires tyrosine or phenylalanine in the protein molecule adjacent to the peptide bond to be split.

In addition to these endo-peptidases there are various exo-peptidases present in the small intestine. These exo-peptidases act on peptides formed by the action of pepsin, trypsin and chymotrypsin. Some of them, carboxy-peptidases, split amino acid residues from peptides beginning at the acid end of the peptide molecule. Tyrosine or phenylalanine residues in the peptide chains are required for carboxypeptidase action. Other exo-peptidases split amino acids from peptide chains beginning at the basic (amino) end of the chain. A leucine residue adjacent to the peptide bond to be split is required for activity.

Since much of the material sold by the meat packing industry is sold as human food or animal feed, an understanding of these digestive enzymes is essential to an evaluation of packing house products sold as food or feed. Some proteins, such as hoof, horn, hair (keratins), are hydrolyzed very slowly, if at all, by these digestive enzymes and are therefore of little value for feed. Extensive heat treatment of protein material in the presence of carbohydrate may cause the formation of complexes which cannot be hydrolyzed by the action of the proteolytic enzymes in the digestive system and therefore reduce its feeding value because it is not digested. This same principle may apply to meats processed for human consumption.

The proteolytic enzymes of muscle tissue are of extreme interest in the meat packing industry because of the role that they play in changes which

occur during the storage and processing of meat. Unfortunately our knowledge of these tissue enzymes is not nearly so extensive as our knowledge of the digestive enzymes. However, it is rather generally accepted that these tissue enzymes are responsible for many of the changes which take place in muscle tissue after an animal is slaughtered. When meat is stored, even at refrigerator temperatures, there is a gradual degradation of protein as eveidenced by the liberation of amino and sulfhydryl groups. Also collagen is apparently transformed into a different compound which no longer has the characteristics of the native protein. The muscle fibers undergo a considerable amount of disintegration as the result of these autolytic changes catalyzed by proteolytic enzymes. These changes are shown in Figure 5. Up to a certain point these changes are desirable be-

A   B
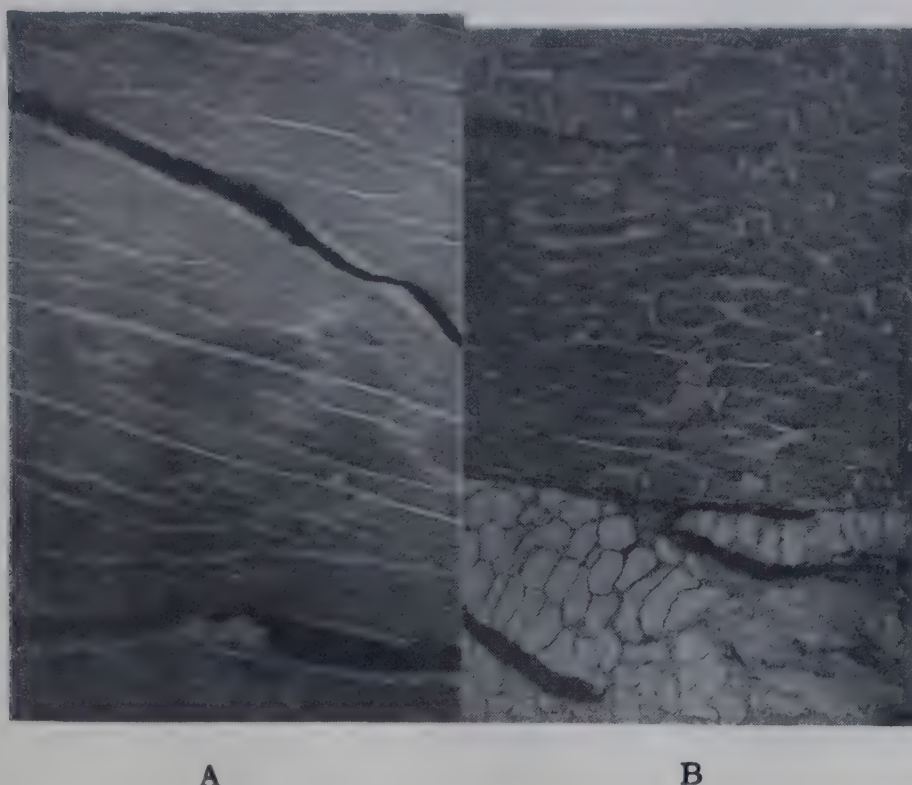
Figure 5. The influence of aging at 35º F. on ribeye muscle. Note the hydrolysis of collagen (black areas) and muscle fiber disintegration in the two weeks aged sample (B) as contrasted to the fresh sample (A) from the same carcass. 49x. (Courtesy of Dr. Hsi Wang, American Meat Institute Foundation.)

cause they result in a tenderizing effect as well as improving the juiciness and flavor. However, not all meat of the same type responds in the same way to aging, either low temperature or high temperature; thus it appears absolutely essential that we obtain additional information on the enzymes involved in order to establish more exact conditions for proper treatment to produce highest quality meats of all types.

Information available on muscle proteinases indicates that they belong to the class of proteolytic enzymes known as "catheptic enzymes." These enzymes occur in such tissues as liver, kidney, and spleen in relatively high concentrations but in muscle tissue in much lower concentrations.

Some, but not all, of these enzymes are activated by such reducing compounds as hydrogen sulfide, cysteine and glutathione. The substrate requirements for activity, as well as optimum pH, temperature, etc., vary widely. For muscle enzymes, the effect of these factors is not known. If we knew the characteristics of these enzymes it might be possible to greatly reduce the aging time required for beef tenderizing by applying some activator or by some other equally simple technique.

There are also several peptidases in muscle tissue. One of these is specific for leucyl-containing peptides with an optimum pH of 8.3. Others split alanyl-containing peptides.

Even before we obtain more detailed and specific information on these proteolytic enzymes of muscle, it is possible to apply our general knowledge of enzymes to problems of meat aging, storing, and processing. Since the rate of any enzyme-catalyzed reaction is greatly increased by increased temperatures, then desirable enzymatically controlled reactions may be greatly speeded up by using higher temperatures. This of course is the principle upon which is based the short time, high-temperature aging of beef. Since meat is such a fine medium for microbial growth however, precautions must be taken to prevent bacterial contamination if the product is held much above the freezing point for any length of time. Also, it must be recognized that not all enzyme reactions are accelerated to the same extent by an increase in temperature. Thus an increase in storage temperature might increase undesirable reactions at a greater rate than it did desirable reactions and yield an overall decrease in desirability. Of course, the reverse might just as logically be true. These enzyme-temperature effects should be carefully considered in smoked meat products where internal meat temperatures in the smokehouse are nearly ideal for high enzymatic activity. Of course, the curing salts used prior to smoking may have inactivated some of the enzymes, but may just have logically activated others.

Since the muscle tissue enzymes are intra-cellular any treatment which destroys cell structure, such as grinding or freezing and thawing, may greatly increase enzymatic activity simply by making it possible for the enzyme to come in contact with an appropriate substrate. Thus ground meat will exhibit undesirable protein decomposition changes much more rapidly than unground meat even under refrigeration where bacterial growth is retarded.

Oxidative Enzymes. Before discussing the importance of specific oxidizing enzymes in meat and meat products, a few general remarks about biological oxidation should be made. First, oxidation, in the true chemical sense, may be simply defined as a reaction in which electrons are lost from the substance oxidized. Thus it is not necessary for oxygen to be present for oxidation to take place if some other electron acceptor is present. Actually, most biological oxidation reactions involve dehydrogenation with some compound other than oxygen acting as the hydrogen acceptor. It is only in the final aerobic stage of oxidation that oxygen becomes necessary as the end acceptor of hydrogen that has been transferred from the original substrate through a whole "bucket brigade" of acceptors. Hydrogen is transferred successively from one acceptor to another under the influence of enzymes known as "Dehydrogenases." For their action some co-enzyme is usually required. The principal co-enzymes for these reactions are riboflavin, diphosphopyridine nucleotide, and triphosphopyri-

dine nucleotide.

The second important fact to remember about biological oxidation is that one of the end products is energy. Thus energy relationships are extremely important in any consideration of biological oxidation. When an organism dies, the entire energy balance is changed because the aerobic phase of oxidation can no longer occur in the tissues.

In normal living muscle tissue, carbohydrates are oxidized to carbon dioxide and water to furnish energy. This change occurs stepwise and involves a whole group of intermediate compounds and enzymes to catalyze each step in the reaction as mentioned before. Quite probably the oxidation follows the Krebs citric acid cycle or the Szent-Györgyi four carbon dicarboxylic acid cycle. When an animal is killed, the cycle is broken because blood no longer circulates to furnish oxygen for the aerobic phase of the oxidation. This effects an entirely different set of conditions in the tissue. Glycogen of the tissues still oxidizes anaerobically, but instead of being completely oxidized to carbon dioxide, it is oxidized only to lactic acid. This causes the acidity of the tissue to increase to approximately 5.5 for beef (pork and mutton are slightly more alkaline) as contrasted to a pH of 7.4 in normal, inert muscle of living animals. If the glycogen content of muscle tissue is low at the time of slaughter as the result of lack of feed and exposure to cold, the acidity increase due to enzymatic oxidation of glycogen to lactic acid is much less. Under these conditions of high pH, "dark-cutting" beef is often obtained. Fundamentally then, one may state that "dark-cutting" beef results from low concentration of an enzyme substrate.

Rigor mortis of animal tissue after death also results indirectly from the increased acidity due to lactic acid formation. The mechanism of the reactions leading to rigor mortis is not completely known or understood, but the essential step in the phenomenon is the removal of phosphate from adenosine triphosphate. This is an enzymatically-catalyzed reaction. The enzyme responsible is inactive at acidities less than pH 6.5. When the pH of the tissue after death drops to this point as the result of lactic acid formation, rigor begins and is essentially complete in normal tissues by the time that the pH reaches 6.3. The ATP-ase activity of different types of animal tissue and tissue from different species of animals is not known. An evaluation of the effect of this enzyme activity on meat quality must await additional information.

Summary

Enzymes are proteinaceous, heat-labile catalysts formed by living cells, but active in the absence of cells. Their activity is greatly influenced by temperature, pH, activating or inhibiting compounds and substrate concentration. Usually an enzyme catalyzes only a single type of reaction with a single compound or group of closely related compounds.

Enzymes are important in the packing industry (a) as by-products, (b) as plant or laboratory reagents, and (c) as factors influencing the preservation of meat and meat products. Lipoxidase and lipase bring about undesirable changes in unheated fatty tissue of meat. The proteolytic enzymes hydrolyze proteins to simpler compounds which is essential in the digestion of meat or meat products used for food or livestock feed. The tissue proteolytic enzymes are responsible for some of the desirable changes that occur during the aging of meat, but also produce undesirable putrefactive changes if allowed to remain active too long. The oxidative

enzymes in meat are indirectly responsible for many of the changes that occur in muscle tissue after slaughter of the animal. The enzymatically controlled oxidation of glycogen to lactic acid indirectly affects such phenomena as rigor mortis and the production of "dark-cutting" beef.

<center>XII</center>

<center>PRODUCT CONTROL IN THE OSCAR MAYER & CO.</center>

<center>
Wm. J. Shannon
General Products Controller
Madison, Wis.
</center>

The highly competitive conditions which exist in the meat industry have made the formation of an active Product Control Department an absolute requirement. It is the function of the Product Control Department to aid in the production of top quality products with constant uniformity from the raw materials available in our own company at a cost that will allow these products to be sold with a fair margin of profit to a critical consuming public in the highly competitive market of today. We are also charged with the responsibility of investigating new methods and new techniques in order to assure our progress.

Product control is the control of product-raw materials used, methods of production, packaging, shipping, etc. Quality control from our viewpoint is the control of the various attributes of our products that the consumer desires such as flavor, color, texture, and uniformity. If we were in the machine industry, quality control would involve the statistical evaluation of a machine's output, the use of X, R Charts, etc. I understand that several of the packers are beginning to evaluate their production from the true statistical approach and this is a move in the right direction.

In order to have a better understanding of our product control program, I would like to review the relationship that exists between our department and the major departments of our company.

Production and Product Control

The foundation of product control lies in the specification controlling the production of the item. We devote an appreciable amount of our time to the writing and amending of specifications. As you all realize, the specification is only of value when it is alive and therefore under constant revision as we learn to evaluate more critically the operation in question.

Once a specification has been established, it is the responsibility of the Product Control personnel in each plant to see — first — that its operation is understood by the operating personnel — finally, that it is complied with. It is in this function that we have the greatest trouble. I suppose it is only natural that a man who, for example, considers himself an expert sausage maker would object to taking instructions from someone as new in the industry as I am; however, by careful review and approach to the operation in terms of helping the operator, we have been fairly successful. I will admit that there have been times when the problem has seemed hopeless but believe me, we are making progress.

I would say that of prime importance to the success of this project is

the establishment of the correct relationship between the production super-
visors and the various Product Control personnel. It must be pointed out
at this time that the responsibility of producing quality merchandise rests
with the operating personnel. No matter how elaborate the program or how
many people there are on the Product Control staff, if the operating per-
sonnel do not accept the responsibility of producing products of top qual-
ity, the entire program will fail. Product Control men are not policemen
trying to discredit the operating personnel — rather we are there to help
them solve their problems. Carefully planned educational programs are a
great help in presenting the story of quality and uniformity to the various
members of the Production Department. I have found that when the opera-
tor realizes the overall importance of doing a good job, he will in most
cases cooperate. I will admit that it frequently takes good salesmanship to
establish this point. Our easiest job is the introduction of a new product
into production. We can specify the exact manner in which the item is to
be produced and the operators for the most part accept without question
our specifications. However, when we make a change in wiener production
for example, the operator feels that he has made this item for the past
twenty-five years and knows all there is to be known about the operation.
Yes — it takes salesmanship.

New methods as well as new equipment are constantly being checked
and studied by both Product Control and Production personnel. Our man-
agement is interested in the new way and in the improvement of product.
Of course, the constant "trouble shooting" or "fire fighting" is a daily rou-
tine for everyone in the entire organization.

Our outstanding Product Control Laboratories are the real tools for
solving many of the complex problems of production. We have a constant
check on all items we produce as well as on all our raw materials. An ac-
tive laboratory is one of the most important units in a growing product
control organization.

It is our belief that it is better to correct a faulty operation than to
condemn the finished product. With this point in mind, we are developing
a staff of technically trained personnel to study problems of sausage pro-
duction, canning, curing, smokehouse operation, and refrigeration. It is
true that we have an excellent finished production inspection program, but
our major emphasis is on the operations producing the product.

Sales and Product Control
We hold regular weekly meetings with the members of the Sales and
Production departments to review our main products as well as to assure
prompt action on Sales questions or complaints. During the past year our
company has established an excellent program covering new product re-
quests — and these requests may originate in any one of our major depart-
ments. Several times a year we have combined meetings with key person-
nel from all of our plants and at this time the complete line of products
is reviewed in detail. Such a procedure is invaluable in securing uniform-
ity of quality on a company-wide program.

We also have periodic reviews of our main competitors' items as a
means of keeping up with the industry — or should I say ahead of it.

In order that we may have a better picture of the sales problems, the
key men in Product Control also spend a certain amount of time in the
sales field calling on customers with the various members of the Sales

Department. We have found this procedure to be extremely beneficial to both Sales and Product Control members. I personally will always remember my visit to Florida during one of our cold spells!

Provision and Product Control

Our main function with the Provision Department is to maintain close control of our raw material inventories, both fresh and frozen. Our program calls for a weekly review of all our formulas and adjustments are made in line with both inventory position and price of the various raw materials.

Government and Product Control

In view of the fact that all our plants are under the B.A.I. inspection service, we naturally devote a great deal of time to label approvals as well as to special changes in our methods or production. All Federal Government requests are handled through the General Product Control Office and this procedure has aided in our coordination program between our plants. It has been our experience that our label requests take less time when all plants clear through the central office. I might add that the Trade Label Section of the M.I.D. also approves of this procedure.

Many State governments are taking a more active interest in food regulations and this means more work for us. I will admit that some of the requirements are unusual and we believe the industry, as well as the consuming public, would benefit greatly if the Federal regulations were also enforced in the individual states.

Our various Product Control personnel throughout the plants maintain close contact with the B.A.I. inspectors and minor questions are readily settled before they become a serious issue.

Purchasing and Product Control

Both Purchasing and Product Control departments have the same basic goal as far as raw materials are concerned — quality and uniformity at a fair purchasing price. Our department sets up standards for the various raw materials used such as spice, as an example. We also review supply items that are presented to our Purchasing Department by various suppliers to determine if these suppliers are more adequate than the ones we are using at the present time.

Industrial Engineering and Product Control

Our Industrial Engineers are interested in establishing work standards for the various jobs in production and immediately the question of quality becomes an important issue. As you all realize the incentive program does not favor the production of quality products. I suppose it is only natural for the worker to be more interested in his take-home pay than in the quality of the product he produces.

Our company has a constant educational program designed to impress all employees with the importance of the quality of our products. I firmly believe that once the workers are made aware of the overall picture of quality with relation to job security, better products will result.

Cost Accounting and Product Control

It is the responsibility of the Product Control Department to see that

the Cost Accounting Department has the correct yield figures, standard yields, and raw material usage reports. These figures must represent the true picture if management is to make the correct decisions in their policy meetings.

Research Department and Product Control

The coordination between Research and Product Control is indeed positive. The personnel from both departments review their various problems informally and this close contact is beneficial to all. Many of our projects are carried out in the pilot plant with members of both departments working side by side. When research items are being cleared for production, the Product Control Department personnel takes the item over in the pilot plant before moving the operation into production. We have found that the pilot plant transfer of new products has eliminated many of the headaches one normally has with new items in production. You will note I said many of the headaches — not all of them.

It is our opinion that the Research laboratory should be entirely separate and removed from the Control laboratories. The research type of approach should never be hampered by the constant flow of urgent routine problems so characteristic of the Control laboratory.

General Internal Organization of the Product Control Department

I have frequently been asked to describe the general type of personnel we have in our organization. At the present time our group includes food technologists, chemists, chemical engineers, mechanical engineers and bacteriologists, as well as a good balance of the practical men who have worked their way up in our company.

I am interested in building an organization of technically trained personnel to study in detail our various operations. I want our people to use their technical training as a background or a stepping stone to aid them in their studies of the problems peculiar to our business. As most of you know we have few positions that are strictly chemical engineering, for example. In order to give the new man additional working background, I require him to study the A.M.I. correspondence courses, as well as to take part in the various technical meetings. We also arrange for all our new men to tour and study for brief periods of time each of our departments so that he has the overall picture of the company's organization.

I hope that the Product Control Department will some day be the training ground for key men of our Operations Division because I firmly believe that the problems so peculiar to our particular business will best be solved by technically trained personnel.

XIII

METHODS OF FOOD TASTING EXPERIMENTS

L. L. Thurstone
Department of Psychology
The University of Chicago

Methods in Food Tasting Experiments

When I first learned about the food-tasting laboratory at the Quartermaster Corps here in Chicago, I was much interested to see it. I found in that laboratory five booths which were specially designed for experimental work in this area. I volunteered as a subject, and I was seated at a table next to a wall. On the other side of the wall were technicians who placed the food specimens on a small revolving platform. I was presented with a number of specimens of tomato juice. The instructions told me to rank the specimens according to my preferences. These specimens had been stored for different lengths of time at different temperatures in different types of containers. The practical question was to determine whether people can tell the differences and whether they will reject some of these foods. It has been found that the human subject can make discriminations to accept or reject foods with criteria that are not evident in the chemical and bacteriological analyses. In developing new foods it is then an essential preparation to ascertain whether people will eat them. This is called food acceptance research.

It is an interesting circumstance that the laboratory methods for determining sensory limens and hedonic values has here an important practical application. This is the oldest part of experimental psychology, and it is called psychophysics. Traditionally, this field has been known among students of psychology as a very theoretical and dull subject, but it has in recent years found so many applications that it is now coming to be regarded as an interesting aspect of psychological method.

One of the first problems of the food-tasting laboratory is to arrange the procedures to obtain a maximum of information from each subject in the minimum time. In testing a new food product by taste experiments it is desirable to compare different specimens which have been kept at different temperatures for different lengths of time so that it will be known under what latitude the product remains not only edible but also desirable.

One of the controls in the food-tasting booths is the color of the illumination. By light switches the examiner can instantly change the color of the food that is being rated. A piece of butter suddenly looks like a piece of lard, and acceptability might be affected.

A theoretically more interesting problem is that of predicting from the ratings what proportion of the subjects will choose food A when it is offered in competition with foods B and C and D, for example. It is not possible to experiment explicitly with every possible food combination. There are too many. How should the ratings be made so as to enable us to make predictions of this kind? Such a problem is of analytical and practical interest.

Taste experiments are sometimes conducted with an especially se-
lected panel of expert tasters. Such people are selected supposedly be-
cause they excel in the ability to make fine discriminations in tastes and
sometimes because of their familiarity with the particular product. In se-
lecting such panels one should keep in mind that a person who can make
very fine discriminations in one class of tastes is not necessarily super-
ior in tasting different products. In general, it is probably true that a per-
son who has keen taste discrimination tends to excel in all taste discrim-
inations, but this is not a dependable rule for individual cases. A differ-
ent policy is to select a group of tasters who represent the public that is
to be pleased rather than a small group of experts. If the taste experi-
ments are conducted for the purpose of predicting relative sale or con-
sumption for competing flavors or brands, then it would seem best to
work with an experimental population that is truly representative of the
public for whom the product is manufactured. On the other hand, since it
has been found that the human subject can make taste discriminations cor-
rectly even when the chemists and bacteriologists are unable to demon-
strate the differences objectively, the expert taster can be used to advan-
tage. Before a panel of expert tasters are put to work, they certainly
should qualify by objective tests as to their ability to discriminate and as
to the consistency with which they make taste discriminations.

Most of the experimental work in psychophysics has been done with
vision and hearing. Such experiments are usually carried out more easily
with visual stimuli where the subject can see two objects simultaneously.
With auditory stimuli that cannot be done. The stimuli must be perceived
in succession. This is also true in experiments with taste and smell. In
conducting taste experiments one must deal with several difficulties that
are specially annoying. When the subject has tasted one specimen, he can-
not immediately turn to the next specimen. Preferably, he should rinse his
mouth and even then several minutes should elapse before he can taste the
next sample under normal conditions. He may be asked to express his
preferences, or he may be asked to make comparisons as to some partic-
ular taste quality. In the cases of smells, experimental work has the addi-
tional difficulty of adaptation. For some odors continued exposure reduces
the ability to discriminate because of adaptation.

Because of these difficulties in experimental work in taste and smell,
it is essential in any practical problem to arrange the procedure in such
a way that the maximum possible information can be obtained from each
subject with the smallest possible number of separate judgments. Because
of these difficulties one must make some practical compromises. In some
types of psychophysical experiments one can present all of the stimuli in
pairs so that each subject makes a comparison of each pair of stimuli.
For example, a set of ten specimens requires 45 distinct paired compari-
sons. In practice, such a procedure is almost out of the question in deal-
ing with taste.

Another procedure is to give the subject, say three, four, or five sam-
ples and to ask him to arrange them in rank order of preference. If a larg-
er number of specimens are to be tasted, they can be divided into several
small groups. For each group the subject is asked to arrange the speci-
mens in rank order of preference. It is assumed that with a small group
of specimens, say four or five, the subject can keep them in mind well
enough to make dependable judgments of rank order. However, even with

the rank order method the subject ordinarily samples each specimen several times unless the differences are so gross that one hardly needs to conduct taste experiments to determine them.

The best procedure is probably to have a fairly large number of subjects and to ask each subject to sample each specimen only once. We might consider here two variations of this principle. If the subject is asked to sample a fairly large number of specimens by tasting each specimen only once, he may be asked to allocate the specimens to a set of say ten steps. These might be numbered from 1 to 10, and he might be asked to let number 1 represent the most disagreeable while number 10 represents the most agreeable taste. These ten steps would really represent intervals on a subjective scale of taste preference. One method that could be used for this work is called the method of equal-appearing intervals. In this procedure the subject is asked to think of the ten steps as representing subjectively equal increments in taste preference from the worst to the best. After tasting each specimen, he allocates it to one of the ten classes. In another context this experimental method has been used to advantage, but it is probably not the most appropriate method for this type of problem. It has been shown that even when the subject attempts to make the intervals objectively equal, he rarely succeeds in doing so. This can be demonstrated by comparing the method of equal intervals with other psychophysical methods that can be checked for internal consistency.

The method of successive intervals is probably the best experimental method for this problem. In this method the subject samples each specimen only once, and he states his degree of preference in terms of one of a number of short descriptive phrases which are assigned to the successive intervals. There is no assumption that these successive intervals of the scale are in any sense equal. The descriptive words or phrases must, however, be sufficiently distinct so that the subject accepts the rank order of the descriptive phrases. In effect then, the subject expresses his degree of preference or dislike in terms out of eight or ten descriptive phrases. For convenience these descriptive phrases can be denoted by numbers or letters. Instead of having a small number of subjects do the taste experiments, it is probably better to have a fairly large number who represent the true population and to ask each of them to describe his judgment about each specimen in terms of the right or ten successive descriptive phrases. This procedure probably enables us to get the maximum amount of information from the subject in the shortest time.

Each specimen is identified by a code number or suitable name. On the record sheet the subject has a row of eight or ten spaces. Immediately under these spaces he finds descriptive phrases from extreme dislike, some dislike, indifference, some preference, to strong preference. When he has tasted a sample, he merely records a check mark to indicate his rating of the degree of preference. This procedure is very coarse compared with the discrimination he could give if his attention were limited to only three or four specimens. The experimental work with other types of stimuli have indicated that this simple method of successive intervals with only one judgment from each specimen for each subject gives results that are useful in prediction. It is assumed that an experimental population of at least several hundred individuals is available. They should be a random sample of the population for which the food products are intended. It is possible to get a fairly large number of specimens ex-

amined by each subject without undue fatigue. The subjects find it easier to make judgments of this kind than to make the more refined paired comparison judgments that are called for when the purpose is to determine the taste limens of the individual subjects.

This is not the occasion to discuss the psychological scaling problem except to indicate the general nature of that problem. In psychological measurement we differentiate between two types of scales, the objective physical scale and the subjective or psychological scale. The physical or objective scale is the type of scale that we all know. The unit of measurement is some physical unit such as the inch, the gram, or the liter. The subjective or psychological scale represents equal steps in apparent or perceived magnitude as contrasted with the physically measured magnitudes of the stimuli. Sometimes we refer to the physical magnitudes as real magnitudes, but one could insist that the real magnitude is that which the subject actually experiences when he does make the differentiation. Let us consider a very simple example to illustrate the difference between the physical stimulus scale and the psychological scale. Suppose that we place before the subject two standard weights which differ markedly. They might be, say 50 grams and 500 grams. Now suppose that the subject has available a large number of intermediate weights and that we ask him to select one of these weights which seems to him to lie midway between the two standard weights. When he finds a weight that seems to him to lie midway between the two given standard weights, then we can determine the physical magnitude of this intermediate weight. It will then be found that the intermediate weight which seems to the subject to lie midway between the two standards is actually physically closer to the lower weight than to the upper one. On the psychological scale the three weights are equally spaced because the subject perceives the two intervals to be equal. On the physical scale, the three weights are not equally spaced. The lower interval is smaller than the upper one. In this case we expect to find that the psychological scale is a logarithmic function of the physical scale. When we deal with problems of discrimination that are concerned with food acceptance, or indeed with accepting and rejecting other commodities, we are dealing with the apparent magnitudes, the subjective scale, because that is a scale that actually functions when we express preferences among several competing objects. One of the central problems in psychological measurement is to determine the ratio of the successive intervals. This problem has been solved so that we can speak of a subjective metric with a subjective unit of measurement and not merely about a set of rank orders.

There is one concept in psychological measurement to which I should like to draw your special attention because it can have far-reaching effects in many practical problems. The concept is rarely understood by those who have the responsibility for making decisions in which this concept should play a part. I am referring to the concept of discriminal dispersion. The central idea of this concept is relatively simple. I shall try to express it briefly and to indicate some of its curious practical effects. Consider two objects which might as well be food products in the present context. Let these two objects have the same average popularity. This may mean that the two products are equally popular or equally unpopular. We mean that the average scale values for these two objects are the same. Let us suppose that they differ in one important respect, namely, that object A has

wide range of values in the population so that some people are enthusiastic about it whereas others have intense dislike for it. We say that such an object has a large discriminal dispersion. People differ very markedly in the hedonic values that they assign to this object. Let object B have a small discriminal dispersion. This means that nobody is very enthusiastic about it and no one has any violent dislike for it. The range of hedonic values for this object in the population is small. It can easily be seen that two objects can differ in this way in the range of feeling that people have for or against them in spite of the fact that the two objects have the same average popularity. We should expect food products like roast beef to have a rather small discriminal dispersion whereas oysters would have a large discriminal dispersion.

If two objects A and B are presented to a group of subjects with instructions that each subject select only one of the pair, then if the average popularity is the same and if the discriminal dispersions are different, we should expect that half of the population would select each item. However, if we have several competing objects which have the same average popularity and one object which has a large discriminal dispersion, then we would expect a rather curious and startling result. Let us suppose that we have six objects of equal average popularity. Let the object A have large discriminal dispersion, and let the other five objects have vanishingly small discriminal dispersions so that people are fairly well agreed about them. If these six objects are presented to the population and if each person chooses one of the objects, then we should discover that the object A gets 1/2 of the votes while the other five objects get only 10% each. In this example I am assuming for illustrative purposes that the preferences for these single items are uncorrelated. It is quite likely that some of the errors in the prediction of elections have been caused by the striking effects of differences in discriminal dispersions. The theory of this problem is very much the same whether we are considering the popularity and dispersions of a group of political candidates or of any other competing objects that are to be chosen.

If we have a record of preference judgments from an experimental population about each one of a large number of foods which might be listed merely by name, then it should be possible to predict the proportion of that population which will select each item as its first choice when it is presented with any given set of alternatives. For example, if we have obtained several hundred preference records with the method of successive intervals for a list of foods, then if any arbitrary list of four, five, or six of these foods is presented for choice, it should be possible to predict the proportion of the subjects who will select each of those foods. This kind of prediction has been made on other types of material, and there should be no reason why the same principle should not be extended to the prediction of choice in foods. Here also the concept of discriminal dispersion plays an important role which market research men could make use of if they knew about it.

If we have the problem of presenting, say five objects out of a total list of 50 objects with the purpose of maximizing the number of individuals who will select one of the five, then there is a fairly definite analysis that should be made. The first impulse is usually to select the five most popular objects from the whole list of fifty and to present these five, but that is the wrong answer. Although this is the wrong answer, I suspect that

such a solution is often attempted. The number of choices from a popula-
tion depends on the dispersions of hedonic values and also on the corre-
lations among these items. It usually happens that those who prefer item
A are more likely to prefer also some other item, say B, and that those
who prefer item C might be those who dislike item E and so on. Merely
to select the five most popular items from the total available list of fif-
ty usually means that many persons will find several items that please
them so that they have difficulty in making up their minds, while other
people will find nothing that pleases them in the presented list of five ob-
jects. A better solution can be described briefly. We start assembling the
required list of five objects by selecting the most popular single item A
from the available set of fifty objects. We remove from the experimental
population all those individuals who did choose this object A as their
first choice. Now we ascertain the most popular object B in the remain-
der of the experimental population. Object B is then added to the collec-
tion. Again we remove from the survey population all those individuals
who did select B as their first choice. Now we ascertain the most popular
object C in the remainder of the survey population. After removing all of
the members of the survey population who did select object C as their
first choice, we might discover that most of our survey population has
been accounted for. If this should happen, then we would have discovered
not only which objects to present to maximize acceptance, but we could
also discover the number of objects that are necessary to attain the re-
quired degree of acceptance. That required degree of acceptance is, let
us say, 90% of the population. This type of solution actually maximizes
the proportion of acceptance. The number of rejections will be smaller
with this solution than if we presented the five most popular objects from
the whole available supply. I am venturing to guess that this principle has
not been applied in dealing with psychophysical problems concerned with
food, but I see no reason why it should not apply in this field.

The traditional psychophysical problems have been concerned with
the establishment of the subjective scale, but we have considered here
briefly an obverse problem; namely, to predict from such a scale what
people will do. This is the problem of prediction of choice. The problem
that we have called the prediction of choice is that of predicting the pro-
portion of the population that will select each particular competing object
when a sample population has expressed its preferences about each of the
separate objects. There is a related problem to which I have recently giv-
en considerable thought. I am calling this problem the prediction of pur-
chase, which is distinct from the prediction of choice. If all of the avail-
able objects are of the same price, then the theorems about the predic-
tion of choice can be applied over the whole range of objects. If these ob-
jects differ in price, we have, of course, different degrees of inhibition
on free choice. The motivation to a purchase can be thought of as the al-
gebraic sum of the hedonic value of the object and the negative hedonic
value of the price. We are working on several psychophysical theorems
concerned with the prediction of purchase which is, of course, a much
more difficult problem than the prediction of the free choice among the
available objects. In these psychophysical problems we are not concerned
with the prediction of total volume because that is for economists to wor-
ry about. Given, however, the expected total volume, it is largely a psy-
chophysical problem to ascertain how the total volume is divided among

competing objects. It seems quite likely that psychophysical theories will find useful application in the solution of important problems of this kind. I see no reason why economics should not be developed along these lines as an experimental science.

In this paper I have tried to describe briefly the nature of some of the difficulties of experimental work on taste and smell with special reference to judgments of preferences. The conventional psychophysical methods which involve paired comparison seem to be less desirable in this field than the more general method of successive intervals even though this method does not claim to be so refined as the traditional methods. If our purpose is to ascertain the frequency distributions of hedonic values of different foods, then it seems best to use as experimental subjects random samples from the population for whom the foods are manufactured. It seems best also to obtain in a similar manner a single judgment from each subject about each one of a large number of foods presented, either by name or by actual specimens, than to confine attention to the work of a small group of expert tasters. In the inevitable practical problems of predicting consumption among competing food items, it should be profitable to take into consideration the discriminal dispersions as well as the inter-food correlations. These correlations can also be obtained from the data that are assembled by the method of successive intervals. The expert food taster still has an important function to supplement other laboratory analyses.

A central theme in food tasting experiments and in survey questionnaires about food preferences is that of maximizing the useful information that can be obtained with minimum effort. The practical problems can be classified in several groups. First is the problem of determining the effect of various conditions of manufacture and storage and containers on food acceptance. Another problem is the prediction of relative volume among different items as determined by experiments on food acceptance. Another problem is that of combining food items so as to maximize acceptance among competing items which differ in discriminal dispersions. It seems likely that formal experimentation in this field can produce more significant and useful results than simple frequency counts at the descriptive level.

XIV

SOME RESULTS OF STUDIES ON FREEZING
AND FREEZER STORAGE OF MEATS

R. L. Hiner
United States Department of Agriculture
Beltsville, Maryland

Freezing and freezer storage make possible a more even distribu-
tion of "fresh" meats that will remain safe for consumption throughout
the year. However, the successful freezing and freezer storage of these
meats is not as simple as it may sound. We all know that just because a
sample or cut of meat is hard to the touch, it is not necessarily of the
highest quality, or that it is always equal in quality to what it was as a
fresh, unfrozen cut. We must recognize at the beginning that except for
improving tenderness under certain conditions, freezing does not improve
the quality of the meat. The most we can hope to do through research is
to devise ways and means of retaining the quality that was originally pres-
ent. The purpose of this paper is to bring to your attention some of the
findings that have been reported from the Agricultural Research Center,
Beltsville, Maryland.

Structure of the Muscle

To better understand the nature of meat and what happens to it dur-
ing freezing it might be well to consider the structure of a normal mus-
cle and its fibers. Muscle fibers, such as make up a voluntary muscle,
are rounded or oval in cross-section but vary in length and diameter.
Each fiber is made up of contractile muscle substance that is enclosed
in a tightly adherent sheath — the sarcolemma. The composition of the
muscle substance includes a complex fluid, long fine fibrils (myofibrils)
that run longitudinally, and numerous nuclei usually located just beneath
the sarcolemma. A muscle fiber contains numerous dark and light bands
or discs which give it a striated appearance.

A large group of muscle fibers bound together by interstitial connec-
tive tissue forms a muscle bundle, and a group of muscle bundles makes
up a muscle. Interspersed within the connective tissue are fat globules.
A normal muscle contains approximately 60 to 75 percent of water, most
of it being within the fibers. The percentage of moisture is inversely as-
sociated with the percentage of fat present in the muscle.

Rate of Freezing

During the freezing process the water present in the meat juice tends
to freeze out in the form of ice crystals, which are pure water. The size,
frequency, and location of these ice crystals depend on the rate at which
the meat is frozen, whereas the amount of water frozen as ice crystals
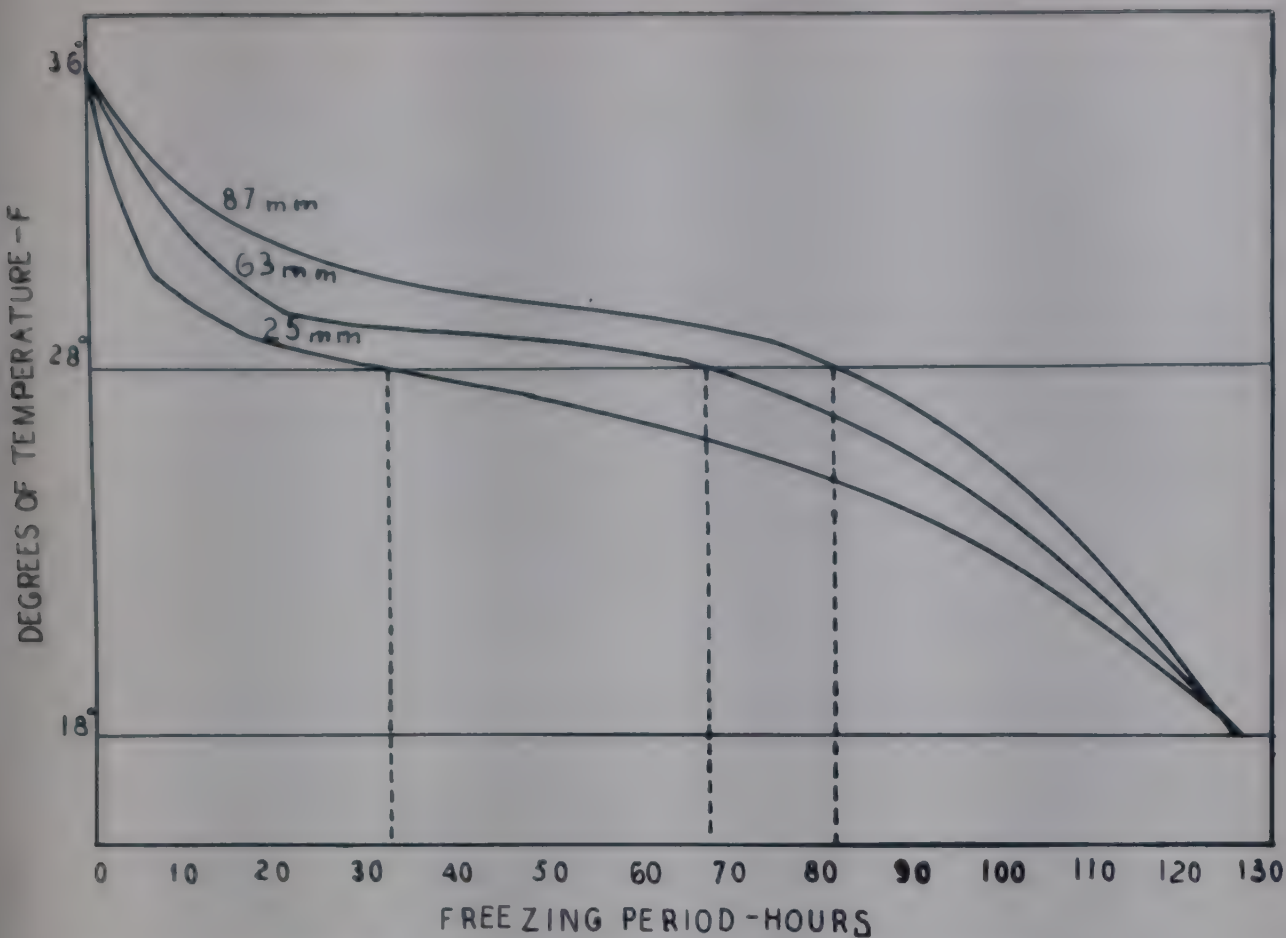depends on the final freezing temperature, as reported by Moran (1).

Figure 1. — Temperature decrease of beef rounds at distances of 25, 63 and 87 mm. from surface in +18° F. freezers.
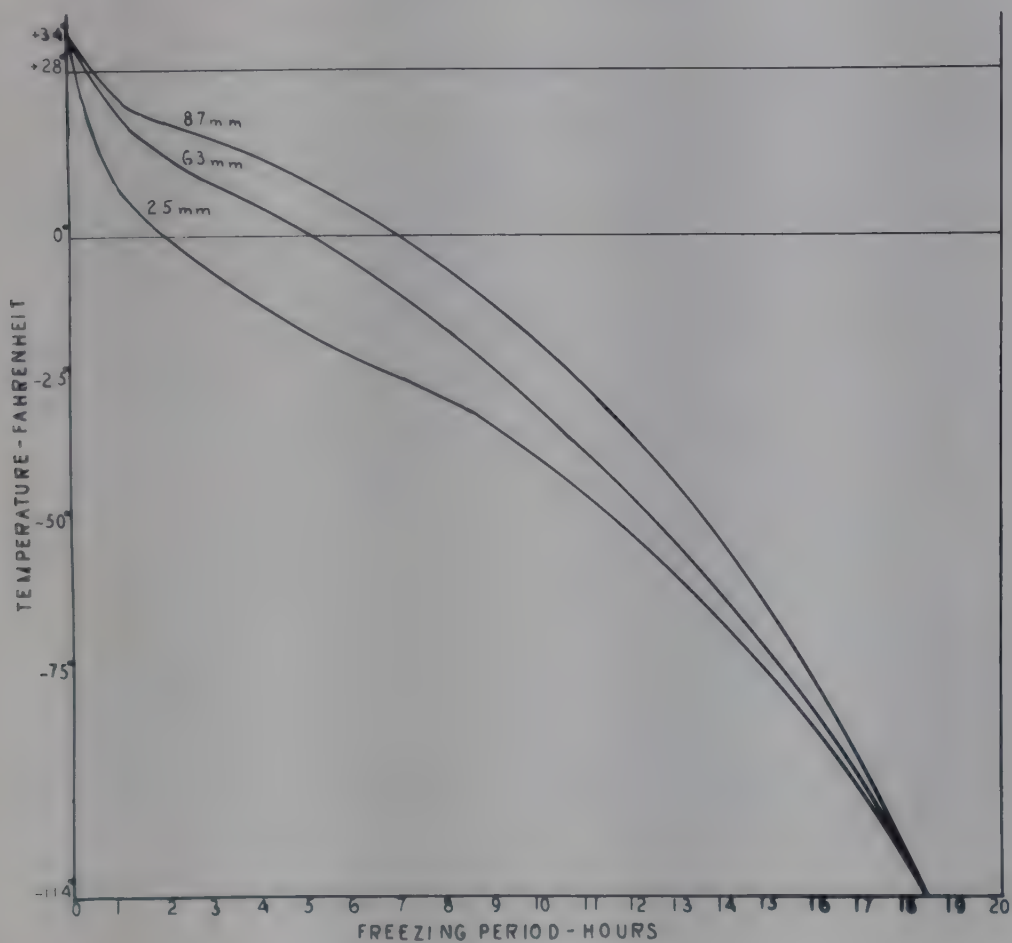

Figure 2. — Temperature decreases of beef rounds at distances of 25, 63 and 87 mm. from surface in -114° F. freezer.

The rate at which meat will freeze depends almost entirely on the method used, size of cut, and temperature of freezer, according to Meek and Greene (2), DuBois and Tressler (3), and Tressler and Evers (4). Ramsbottom and associates (5) report that meat containing a high percentage of fatty tissue froze more rapidly than that containing very little, grinding beef muscle did not change freezing rate significantly, increased air velocity up 500 feet per minute increased freezing rate significantly, and increase in insulating value of wrapping material decreased freezing rate.

Hiner and Hankins (6) reported that an average of 126 hours was required for the internal temperature of beef rounds to be lowered to 18° F. in still air at 18° F. The outside 25 mm. decreased most rapidly to the point of crystallization, or 28° F.; but further freezing was retarded until the center portion had reached a similar temperature. Thus approximately 28 hours were required for the outside portion to reach 28° F. and 98 additional hours to be lowered the next 10°. The reverse of this was true in the case of the center, time requirement here being 61 and 59 hours, respectively. Both inside and outside areas reached the temperature of the surrounding air at about the same time (fig. 1). Similar freezing curves were obtained at other temperatures, except that shorter time difference were noted. For example, at -114° F. the outside 25 mm. was frozen at 0° F. in 15 minutes, at which time a short lag was noted. The inside region require 30 minutes to reach 0° F. and between 18 and 19 hours were required to bring the internal temperature down to -114° F., (fig. 2). The explanation of this difference in the rate of freezing of the inside and outside portions appeared to be that the outside portion froze first and a frozen material, being a good conductor of heat, was kept at a relatively high temperature by transfer of heat from the center.

Freezing in Relation to Tenderness

In 1938, Hankins and Hiner (7) reported that freezing beef loin sample which had been aged 5 days, cut 1-1/2 inches thick, had a tenderizing effect as shown by a decrease in pressure required to shear them. The samples of beef were cut, frozen, thawed, and heated to an internal temperature of 140° F. and tested with the Warner-Bratzler tenderness machine. Paired mates were tested in a like manner except that they were tested for tenderness at the time the others were put in the freezer. The percentage difference varied according to the temperature used in freezing, it being, on the average, 12 percent for the 16 samples frozen at $+20^O$ F. and 18 percent for these frozen at -10° F. Statistical analysis of the data showed that at both temperatures the decrease in shearing resistance due to freezing was highly significant.

This result raised the question of what happens when meat freezes. The present author with Madsen and Hankins (8) reported that on histological examination of the unstained sections of meat, those frozen in still air at 18° were found to contain large ice areas outside the cell walls, the water apparently having been withdrawn from the cell and frozen. No intrafiber ice crystals were visible. Furthermore, the fibers were distorted and bunched into irregular shapes (fig. 3).

Examination of samples frozen at 0° F. showed evidence of intracellular ice crystals, as well as smaller ice areas and crystals than in beef frozen at $+18^O$ F. The first evidence of fiber rupture was observed

94

Figure 3. — Stained longitudinal section of longissimus dorsi muscle of beef after freezing at +18° F. X 150.
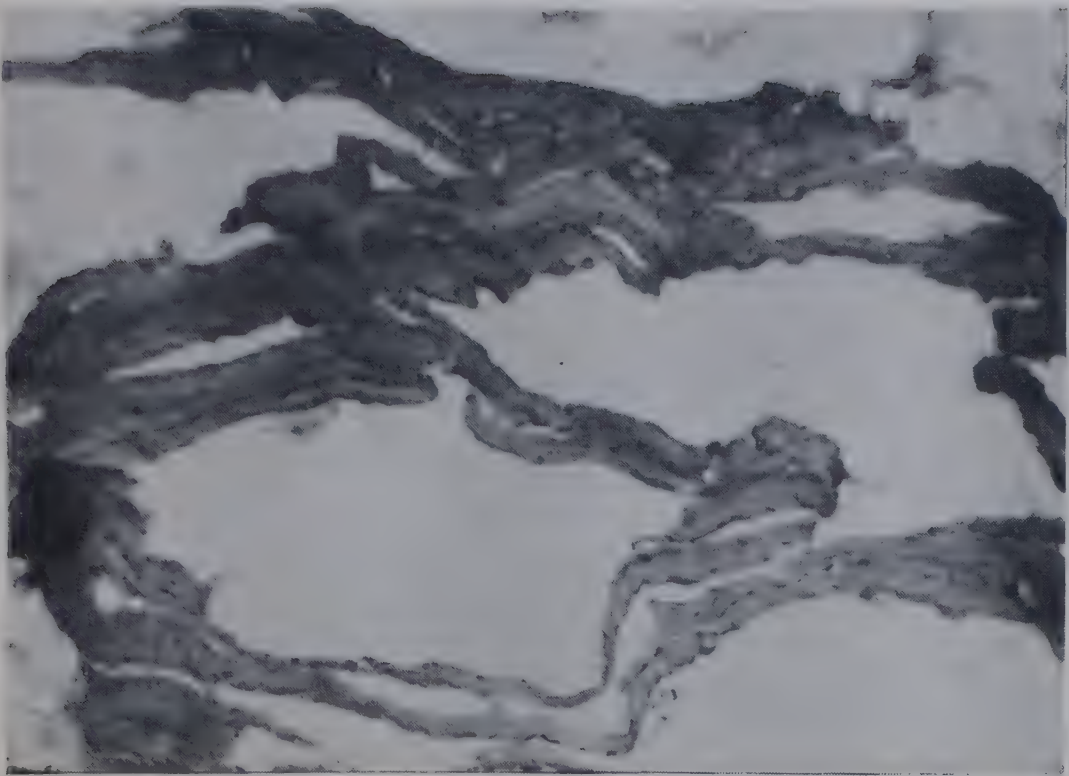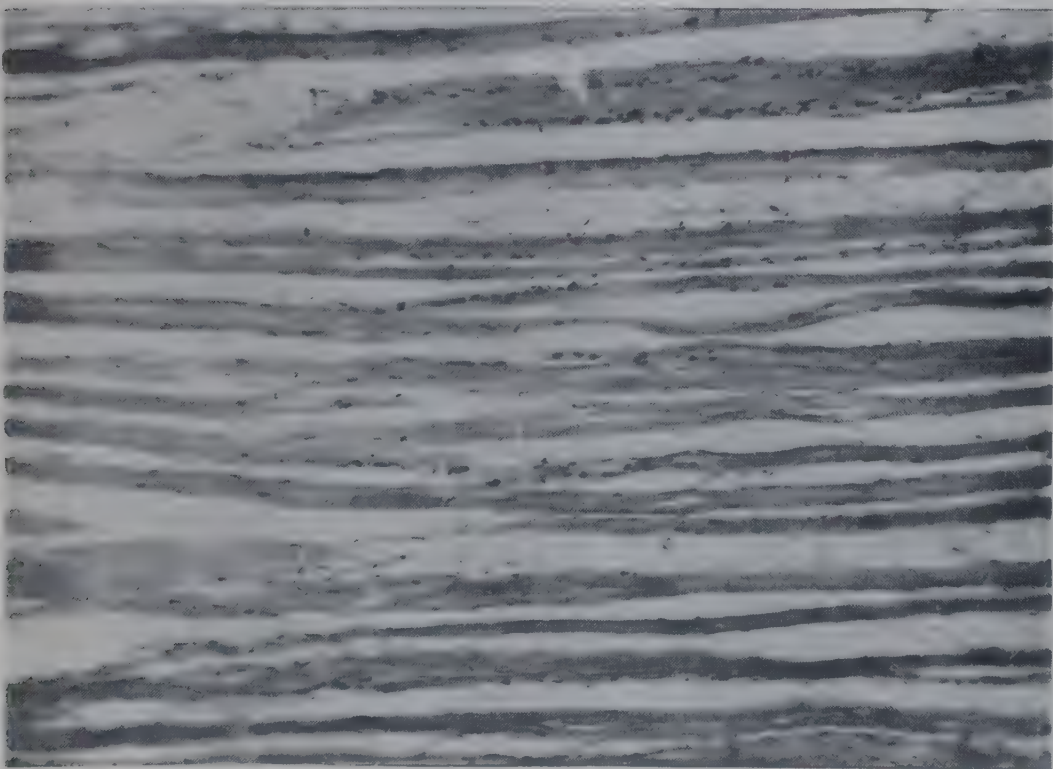

Figure 4. — Stained longitudinal section of longissimus dorsi muscle of beef after freezing at -114° F. X 150.

Figure 5. — Longitudinal section of semimembranosus muscle of beef frozen at +18⁰ F. Sample taken between 0 and 25 mm. from surface.

Figure 6. — Longitudinal section of semimembranosus muscle of beef frozen at +18⁰ F. Sample taken between 100 to 125 mm. from surface. X 150

in beef frozen at -10°. Again the size of the ice crystals was reduced but their frequency and distribution, both inter- and intra-cellularly, were increased. Histological examination of samples frozen at -40° F. showed further decrease in crystal size with increase in number of ice crystals and extent of fiber splitting. Samples frozen at the lowest temperature, -114° F., were split longitudinally, due undoutedly to very rapid freezing. The ice crystals were very much smaller and more numerous and were located almost entirely within the fibers (fig. 4).

Examination of stained sections confirmed the above observations and, in addition, indicated that fiber splitting started at 0° F., and that as freezing temperatures were lowered, or the rate of freezing increased, precipitated proteins and nuclear fragments became more and more in evidence.

A later study, reported by Hiner and Hankins (6), on the freezing of beef rounds, showed on histological examination that ice crystals approximately 25 mm. from the cut surface were distinctly larger and resulted in more fiber bunching than was observed in those approximately 12 125 mm. from the surface (figs. 5 and 6). The ice crystals in sections examined at intermediate levels of 50, 75, and 100 mm. from the surface were progressively smaller and less bunched.

Examination of beef rounds frozen at 0°, -15°, and -40° F. again showed relatively large ice crystals 25 mm. from the cut surface, and they became smaller toward the center. However, at each 25 mm. distance the ice areas were smaller than those frozen at the next higher temperature. Fiber ruptures as well as smaller and more numerous ice formations were observed at -15ᴸ and below.

Freezing was so rapid at -114° F. that the smaller ice crystals and more extensive fiber rupture were found near the surface, with the largest ice crystals 25 mm. from the cut surface. However, at each succeeding interval of distance, the ice crystals became smaller and more frequent (figs. 7 and 8).

The temperature and not the rate of freezing apparently influenced the tenderness of the meat, as shown in table 1 and figures 1 and 2. The beef rounds frozen at any of the three temperatures required less pressure to shear at four distances than the unfrozen rounds. Furthermore, those frozen at the highest temperature required the most pressure to shear and those at the least temperature required the least.

Frequently the higher grades of Choice and Prime beef are aged from 4 to 6 weeks to insure their being tender and having a distinct flavor. This practice is not only time consuming but also wasteful, as the meat is generally covered with mold and requires considerable trimming. Knowing that freezing does have a tenderizing effect, the next step was to determine whether freezing alone or in combination with aging was as effective as aging, and if so under what conditions. Hiner and Hankins (9) reported that beef samples aged 5 days followed by freezing at -10° F. were more tender than similar samples aged 35 days at 34° F. At each aging period of 15, 25, and 35 days, freezing beef at -10° F. had a tenderizing effect. However, after 15 days of aging, the tenderizing effect due to freezing was less apparent. Other similar beef samples frozen at +20° F. were intermediate in tenderness and at the 25 and 35 day aging periods were practically equal to those frozen at -10° F. (fig. 9). Therefore to minimize the danger of spoilage

97
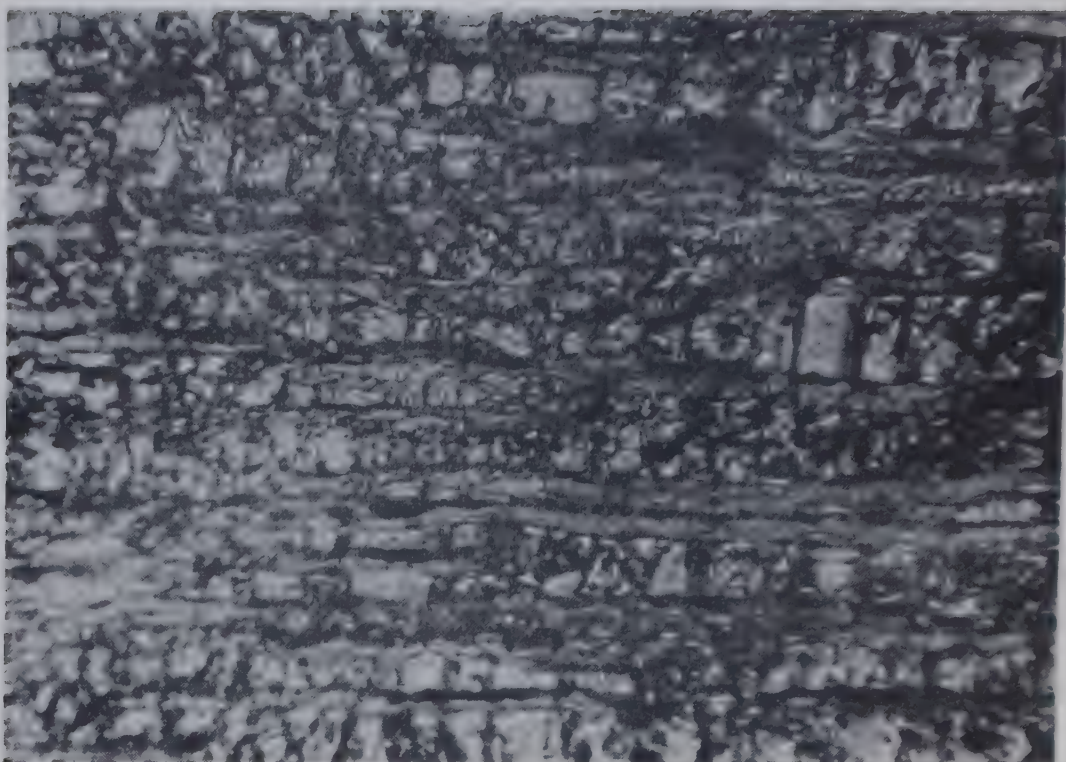
Figure 7 — Longitudinal section of semimembranosus muscle of beef frozen at -114° F. Sample taken between 0 and 25 mm. from surface. X 150
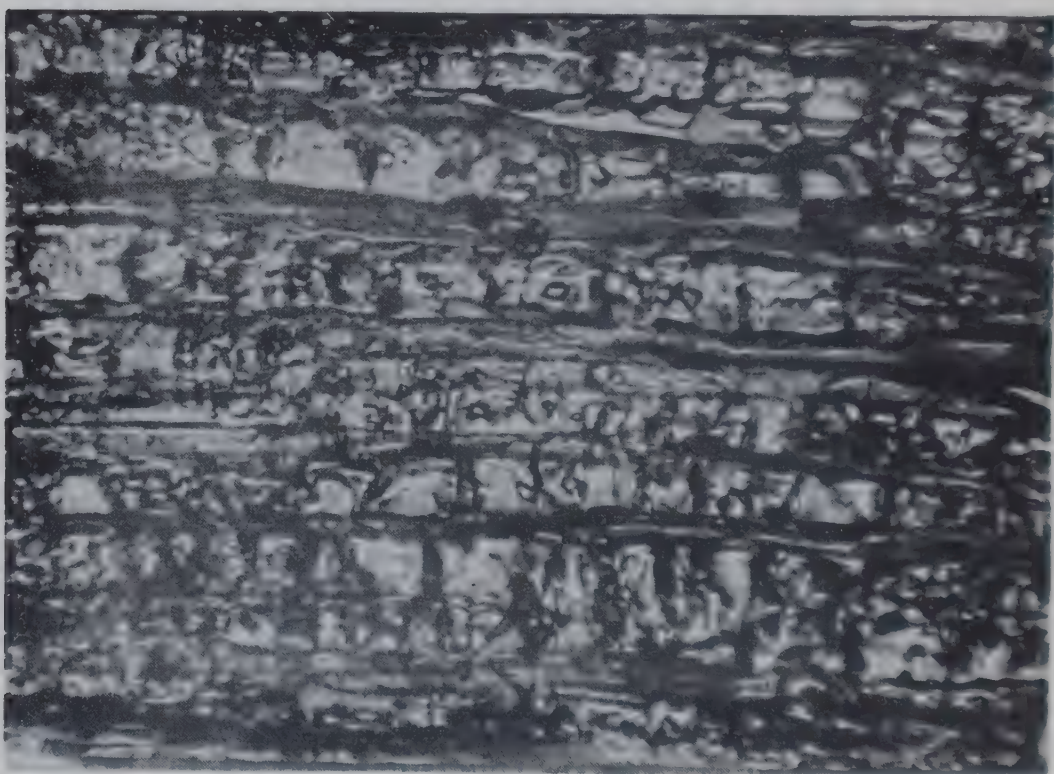
Figure 8 — Longitudinal section of semimembranosus muscle of beef frozen at -114° F. Sample taken between 100 and 125 mm. from surface. X 150

Table I -- Average Resistance to Shearing (Tenderness) of Beef as Affected by Temperature of Freezing and Distance from Cut Surface.

Temperature of freezing °F	Sample number	Number of pairs of round	Distance of sample from cut surface of round mm.	Resistance to Shearing		Decrease in resistance to shearing per cent
				Unfrozen sample pounds	Frozen sample pounds	
18°	1	7	0-37	19.10	15.91	16.55
	2		37-74	19.55	16.86	13.54
	3		74-111	19.55	16.60	14.94
	4		111-148	19.73	17.05	13.56
0°	1	7	0-37	18.84	15.66	16.89
	2		37-74	18.46	15.76	14.39
	3		74-111	18.48	15.21	17.65
	4		111-148	17.81	14.48	18.63
-114°	1	7	0-37	18.63	14.44	22.14
	2		37-74	17.66	14.05	19.81
	3		74-111	18.19	13.77	23.92
	4		111-148	18.35	13.42	26.78

from aging and yet obtain the largest tenderizing effect, a 15-day aging period followed by freezing at -10⁰ F. would appear advisable for Low Good grade beef.

In continuation of the study of freezing and its relation to tenderness, beef samples were frozen at -10⁰ after 5 days aging, thawed at 45⁰ F. and immediately refrozen at -10⁰ F. (10).The samples that were thawed and refrozen were more tender than similar samples frozen once, thawed and tested. The combined effect of freezing, thawing, and refreezing was a 25 to 30 percent reduction in resistance to shearing. However, refreezing of meat has not been and is not now a recommended procedure.

All the results mentioned were obtained from beef samples that were thawed and tested within 1 to 5 days after the sample had reached the prescribed freezing temperature. These results led to the question of what happens if beef samples are left in storage for a longer period . A study just completed (table 2) indicates that the tenderizing effect produced in freezing is soon lost in storage. A total of 144 samples were used in this study. They were obtained from 12 pairs of beef short-loins from carcasses that were approximately Low Good in grade. The samples were aged 10 days; representative samples were tested for tenderness and the remainder frozen at 0⁰ F. As soon as the samples had reached 0⁰ F. representatives were again removed, thawed, and tested. At the end of 12, 24,36. and 48 weeks other representatives were removed and tested. In all, six groups of samples were tested, each group being represented by 24 samples. Freezing overnight, thawing, and testing resulted in a 9.3 percent reduction in pressure required to shear the samples, whereas those stored 12 weeks required 2.1 percent more
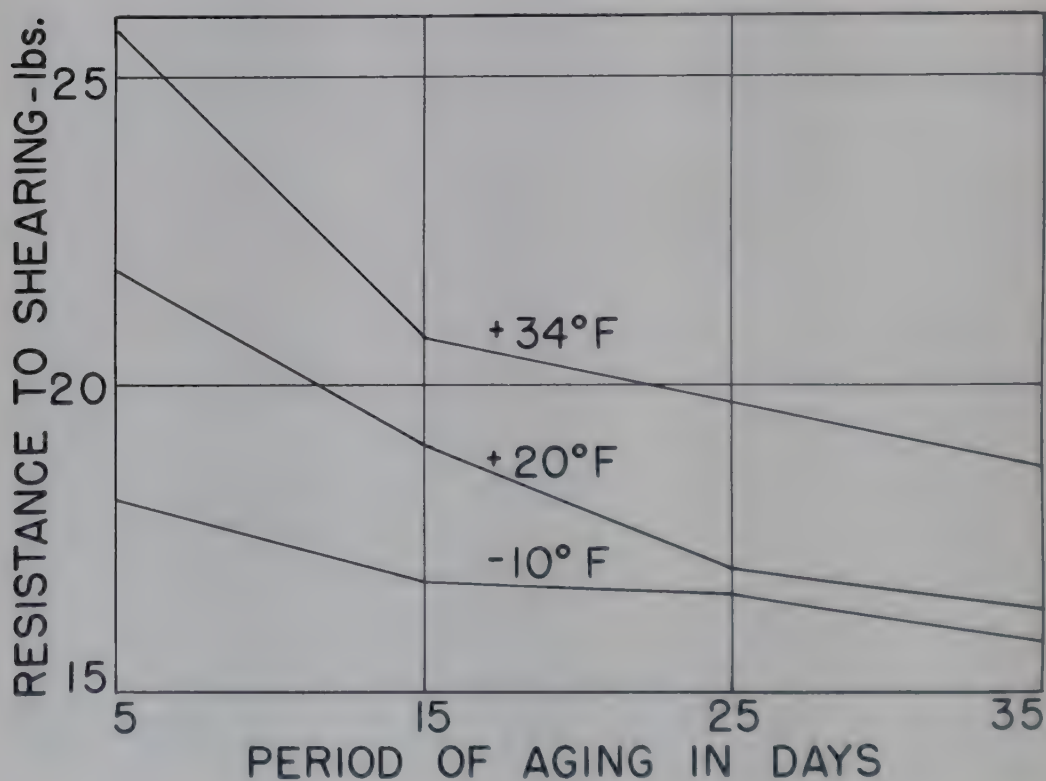
Figure 9 -- Resistance to shearing (tenderness) of beef as affested by aging and aging followed by freezing.

100

Table II — Average Weight of Beef Samples, Resistance to Shearing
(Tenderness) and Percentage Difference in Tenderness of
Beef Samples Frozen and Stored at 0° F.

Storage period weeks	Number samples	Weight fresh grams	Weight frozen grams	Weight thawed grams	Resistance to shearing pounds	Difference between frozen and unfrozen samples in resistance shearing per cent
Fresh	24	496.6	---	---	13.08	---
0	24	505.2	495.1	497.8	11.87	-9.27
12	24	496.8	487.1	486.0	13.35	+2.05
24	24	497.2	487.2	486.3	12.57	-3.89
36	24	510.7	499.9	497.1	12.50	-4.47
48	24	501.8	493.0	488.4	12.82	-2.05

Table III — Mean Weights of Fresh, Frozen and Thawed Pork Samples,
Total Loss in Freezing and Thawing and Resistance to Shear-
ing (Tenderness) as Related to Temperature of Freezing.

Temperature of freezing °F.	Number samples	Weight fresh grams	Weight frozen grams	Weight thawed grams	Total loss per cent	Resistance to shearing pounds	Decrease in resistance to shearing per cent
33-35°	8	648	---	---	---	18.5	---
+18°	8	649	629	621	4.41	15.6	15.68
0°	8	638	631	623	2.49	13.6	26.49
-114°	8	635	632	625	1.57	12.6	31.89

than the unfrozen samples. At the end of 24, 36, and 48 weeks of storage, 3.9, 4.5, and 2.1 percent respectively, less pressure than that of the unfrozen sample was required. This change in tenderness cannot be attributed to dehydration as all the samples were sealed in moisture-vapor-proof wrapping as soon as frozen and had practically no weight losses.

Tenderness in pork and lamb does not receive the attention that it does in beef. This may be due to the fact that both of these types of meat are from younger animals. However, similar tests have been made with them. Lamb legs frozen after 7 days' aging at -10° F., thawed, then heated to an internal temperature of 149° F., and tested mechanically were found to require 24 percent less pressure to shear than paired mates frozen at $+18^O$ F. (11)

Pork loin samples, 4 inches in length, were cut and divided into four groups of eight each. On the fifth day after slaughter, one group was tested for tenderness, after heating to an internal temperature of 165° F. The other three groups were frozen, one at $+18^O$ F., another at 0° F., and the third at -114° F. After freezing they were thawed and tested in a similar manner to the unfrozen samples.

Table 3 shows that in all cases freezing had a tenderizing effect. At $+18^O$ the tenderizing effect was statistically significant, whereas at 0° the difference was highly significant and at -114° F. very highly significant. However, the difference between 0° and -114° was not statistically significant (12).

Palatability in Relation to Freezer Storage

A troublesome factor in the storage of frozen meats is desiccation or, as it is often termed, "freezer burn." This is brought about through sublimation of the ice crystals in the outside areas of the meat and can be controlled by suitable protection from the atmosphere. Attempts to reconstitute the desiccated area by defrosting in cold water have not resulted in complete success (13). The meat so treated, although considered slightly more juicy, had a less desirable flavor of lean than paired samples that had been wrapped in cellophane.

The aging of meat prior to freezing and freezer storage allows considerable changes to take place within the lean and fat portions. For example, beef samples that were cut and frozen five days after slaughter were more palatable at the end of 48 weeks' storage than similar samples aged 10 to 14 days (14) before freezing.

The fat present in meat is a major consideration in successful freezer storage. Fat is not only present on the outside of the sample but also inter- and intra-muscularly. Undoubtedly it plays a major part in determining the flavor of meat, and changes in fat due to oxidation are probably the most important single factor responsible for this quality loss in freezer storage. During the past several years studies have been conducted to determine the nature of the changes brought about by the presence or absence of oxygen. These studies have been made on pork, beef and lamb at freezing and storage temperatures of 0° and 18° F. In addition to study of the extremes of complete absence of oxygen and no protection, partial protection was represented by two methods, half of the samples being protected by cellophane and the other half dipped in melted lard. Complete absence of oxygen was accomplished by placing the samples in tin plate cans and sealing under 28 inches of mercury, followed

Table IV -- Average Weight Loss of Beef Samples During Freezing, Storage and Thawing as Affected by Methods of Protection.

Storage conditions	No. of samples	Days aged	Weight loss at - 12 wks. per cent	24 wks. per cent	36 wks. per cent	48 wks. per cent.	82 wks. per cent
First Test 0° F.							
Exposed	18	7	7.89	13.03	18.67	16.903/	27.45
Vacuum packed	18	7	0.00	0.00	0.00	0.00	0.00
Second Test 0° F.							
Exposed	9	20	5.861/	6.202/	---	13.27	---
Vacuum packed	9	20	0.00	0.00	---	0.00	---
Cellophane	9	20	1.49	1.85	---	1.73	---
Lard dipped	9	20	1.71	1.37	---	1.76	---

1/ 15 weeks storage; 2/ 31 weeks storage; 3/ 54 weeks storage.

Table V – Average Weight Loss of Pork Samples During Freezing, Storage and Thawing as Affected by Methods of Protection.

Storage conditions	No. of samples	Days aged	Weight loss at -				
			12 wks. per cent	24 wks. per cent	36 wks. per cent	48 wks. per cent	82 wks. per cent
First test 0°F.							
Exposed	15	5	8.01	10.96	15.76	15.15 3/	19.17
Vacuum packed	15	5	0.00	0.00	0.00	0.00	0.00
Second test 0°F.							
Exposed	16	7	5.61	10.32 1/	11.51 2/	13.92 4/	---
Vacuum packed	16	7	0.00	0.00	0.00	0.00	---
Cellophane	16	7	2.02	2.13	1.72	2.09	---
Lard dipped	16	7	0.53	0.90	0.56	1.08	---
Third test							
Storage temperature 0°F.							
Exposed	12	8	4.66	4.10	7.98	14.67	---
Vacuum packed	12	8	0.00	0.00	0.00	0.00	---
Cellophane	12	8	1.59	1.12	0.72	3.58	---
Lard dipped	12	8	1.18	0.68	2.12	2.27	---
Storage temperature +15°F.							
Exposed	12	8	7.90	14.66	17.40	22.28	---
Vacuum packed	12	8	0.00	0.00	0.00	0.00	---
Cellophane	12	8	2.50	3.03	2.76	5.40	---

1/ 31 weeks storage 2/ 39 weeks storage 3/ 54 weeks storage 4/ 51 weeks storage

Table VI – Average Weight Loss of Lamb Samples During Freezing, Storage and Thawing as Affected by Methods of Protection

Storage conditions	No. of samples	Days aged	Weight loss at -			
			12 wks. per cent	24 wks. per cent	36 wks. per cent	48 wks. per cent
First test 0°F.						
Exposed	16	13	12.03	15.28	16.10	17.38
Vacuum packed	16	13	0.00	0.00	0.00	0.00
Cellophane	16	13	0.00	1.11	0.05	0.53
Second test						
Storage temperature 0°F.						
Exposed	16	8	12.08	20.03	19.46	23.10
Vacuum packed	16	8	0.00	0.00	0.00	0.00
Cellophane	16	8	0.81	1.68	0.89	1.67
Storage temperature +18°F.						
Exposed	8	8	24.48	39.07	41.29	40.32
Vacuum packed	8	8	0.00	0.00	0.00	0.00
Storage temperature -114°F.						
Exposed	4	8	1.17	3.96	---	---
Vacuum packed	4	8	0.00	0.00	---	---

by freezing. The samples protected by cellophane were wrapped tightly to exclude as much air as possible, then heat sealed. The samples protected by a coating of lard were frozen, then dipped in melted lard and allowed to remain in it for a few seconds.

Tables 4, 5, and 6 show that there was no weight loss from any of the samples vacuum packed. Also there was little weight loss during storage of samples protected by cellophane or dipped in lard. However, there was considerable weight loss of samples stored unprotected, which appeared to be governed by two factors — first, temperature of storage, the higher storage temperature resulting in more shrinkage; and second, the type of meat. Pork, due probably to higher inter- and intra-muscular fat content, shrank slightly less than beef or lamb.

Organoleptic tests of similar samples of pork, beef or lamb at various intervals of storage showed that the principal change was in flavor of fat (tables 7, 8 and 9). The desirability of the flavor of lean appeared to be associated with the condition of the fat, especially in pork. Beef and lamb, with relatively low intramuscular fat content, showed little change in desirability of flavor of lean with increased storage time.

We at the Agricultural Research Station feel that frozen meat has lost little of its original quality as long as the flavor of fat is "moderately desirable" or better. On grading scale of 1 to 6, "moderately desirable" has a score of 3.50. Therefore, when the rating for desirability of flavor of fat falls below this score, the cut is considered to have lost its original or fresh meat quality.

On this basis it was found that for any method of protection except vacuum packing, meats stored at 0° F. were definitely superior to those stored 15° or 18° F., especially pork, probably due to the active lipoxidase present in pork tissue. Pork samples stored at 15° or 18° F. were unpalatable in less than 12 weeks when stored unprotected or wrapped in cellophane, whereas those packed in vacuum in tin plate cans were still in excellent condition after 48 weeks. At 0° F. pork samples stored in vacuum for 82 weeks were as good as similarly exposed samples at the same temperature for only 12 weeks.

Other studies with beef and lamb samples resulted in the same type of changes, although the difference did not become apparent as rapidly.

The decrease in desirability of flavor of lean appeared to be primarily due to fat breakdown, as indicated by chemical analysis of the lean tissue of pork, beef and lamb in which there were no significant changes in pH, titratable acidity and percentage of ammonia nitrogen. However, other chemical tests showed that soluble nitrogen progressively decreased, due probably to denaturation, but there was no significant difference between the various storage conditions, except in the desiccated area in those samples exposed to air.

Changes in the percentage of free-fatty acid (as oleic) were small at 0° F. At 15° and 18° F. storage pork and lamb showed some increase in in free-fatty acid. Fat peroxides and fluorescence increased throughout storage, vacuum packed samples being lowest at all periods of testing. As expected, fat hydrolysis was more advanced at $+15^O$ and 18° F. than at 0° F.

A freezer storage temperature that varies little appears to be important. This was shown by tests in which beef samples were frozen at 0° and 16° F., then wrapped and heat sealed in high grade cellophane and

Table VII — Committee Scores for Palatability of Roasted Pork Samples aged 5 Days at 34º F. before Freezing and Storage 1/

Storage Conditions	Intensity		Tenderness	Juiciness		Desirability	
	Flavor	Flavor		Quality	Quantity	Flavor fat	Flavor lean
12 weeks							
Exposed 0ºF.	4.78	4.89	4.78	3.89	3.89	4.11	4.78
Vacuum packed 0ºF.	4.22	5.11	4.78	4.22	3.67	5.00	5.44
25 weeks							
Exposed 0ºF.	3.89	5.22	5.67	4.56	4.89	4.22	4.67
Vacuum packed 0ºF.	3.56	4.89	5.44	4.67	4.56	5.00	5.22
36 weeks							
Exposed 0ºF.	4.83	4.92	4.75	4.33	3.45	3.00	4.67
Vacuum packed 0ºF.	4.50	5.00	5.17	4.17	4.17	4.75	4.67
54 weeks							
Exposed 0ºF.	5.22	5.00	5.11	4.33	3.44	2.44	4.67
Vacuum packed 0ºF.	4.44	5.00	5.33	4.33	3.89	4.89	5.33
82 weeks							
Exposed 0ºF.	5.22	5.11	5.22	4.00	4.33	2.00	3.22
Vacuum packed 0ºF.	4.89	5.22	5.89	4.22	3.56	4.11	4.44

1/ Scores range from 1 to 6, the latter being a perfect score.

Table VIII -- Committee Scores for Palatability of Roasted Beef Samples Aged 7 days at 34° F. before Freezing and Storage 1/

| | Intensity | | | Juiciness | | Desirability | |
| | Fla-vor fat | Fla-vor lean | Tender-ness | Qual-ity | Quan-tity | Fla-vor fat | Fla-vor lean |
Storage Conditions							
Fresh 0° F.	4.75	4.92	5.83	4.50	4.83	5.00	5.42
12 weeks							
Exposed 0° F.	4.75	5.08	4.75	4.25	4.83	4.00	5.50
Vacuum packed 0° F.	4.92	5.17	5.92	4.42	4.33	3.58	5.51
25 weeks							
Exposed 0° F.	4.33	4.83	5.08	3.92	5.00	2.92	4.83
Vacuum packed 0° F.	4.92	5.08	5.75	4.17	4.75	4.58	5.58
36 weeks							
Exposed 0° F.	4.84	4.84	5.08	4.17	4.25	2.42	4.34
Vacuum packed 0° F.	4.84	4.75	6.17	4.42	4.59	4.08	4.92
54 weeks							
Exposed 0° F.	5.00	5.17	4.58	3.75	4.50	3.58	4.67
Vacuum packed 0° F.	4.67	5.33	5.50	3.42	4.25	4.50	5.58
83 weeks							
Exposed 0° F.	5.17	5.17	5.33	4.17	4.50	2.00	2.83
Vacuum packed 0° F.	5.00	5.50	6.00	4.17	4.83	3.50	4.67

1/ Scores range from 1 to 6, the latter being a perfect score.

Table IX -- Committee Scores for Palatability of Roasted Lamb Samples
Aged 8 days at 34° F. before Freezing and Storage 1/

| Storage Conditions | Intensity | | | Juiciness | | Desirability | |
	Flavor fat	Flavor lean	Tenderness	Quality	Quantity	Flavor fat	Flavor lean
Fresh 0° F.	4.75	4.75	5.50	4.33	4.58	5.33	5.33
12 weeks							
Exposed 18° F.	5.17	4.67	6.00	4.33	4.00	3.33	4.83
Vacuum packed 18° F.	5.33	5.17	6.00	4.17	3.67	4.00	5.50
Exposed 0° F.	5.25	4.83	6.33	4.17	4.00	2.92	4.58
Vacuum packed 0° F.	4.67	5.25	5.83	4.00	3.83	4.92	5.33
Cellophane 0° F.	5.42	5.25	5.75	4.25	4.83	3.25	5.08
Exposed -114° F.	5.33	5.00	6.00	4.00	3.83	3.00	5.00
Vacuum packed -114°F.	5.00	5.00	6.00	4.00	4.33	4.00	5.50
24 weeks							
Exposed 18° F.	5.33	5.33	5.00	2.33	2.33	2.00	3.67
Vacuum packed 18° F.	4.83	4.83	5.17	4.17	3.83	4.67	5.17
Exposed 0° F.	5.33	5.25	5.25	3.17	3.42	3.00	3.92
Vacuum packed 0° F.	4.92	4.83	6.08	4.17	4.00	4.42	5.50
Cellophane 0° F.	5.08	5.25	5.67	4.33	4.50	4.33	4.50
Exposed -114° F.	4.33	5.17	6.17	4.50	4.17	4.33	5.67
Vacuum packed -114°F.	5.00	5.00	5.33	4.50	3.50	5.17	5.50
36 weeks							
Exposed 18° F.	6.00	4.83	5.83	3.83	3.50	2.17	3.83
Vacuum packed 18° F.	5.17	5.00	6.00	5.00	5.00	4.00	5.00
Exposed 0° F.	5.42	5.00	5.17	3.92	3.92	2.83	4.33
Vacuum packed 0° F.	5.00	5.08	5.58	4.50	4.58	4.17	5.33
Cellophane 0° F.	5.33	5.00	5.50	4.25	4.50	3.67	5.25
48 weeks							
Exposed 18° F.	6.17	5.33	5.33	3.00	2.33	2.17	3.50
Vacuum packed 18° F.	4.83	4.83	5.83	4.67	5.33	4.50	5.50
Exposed 0° F.	6.00	5.00	4.92	4.25	3.50	2.33	5.25
Vacuum packed 0° F.	4.92	4.92	5.67	4.50	4.83	4.00	5.42
Cellophane 0° F.	4.83	5.33	5.17	4.67	4.58	3.58	5.50

1/ Scores range from 1 to 6, the latter being a perfect score.

stored at 0°, 16°, and at 0° and 16° F. alternated on a 7-day cycle (13). The flavor of fat had changed little after 24 weeks of storage at 0° F. , but at 16° and at alternating temperature of 0° and 16° the change was rapid, especially after 13 weeks. The flavor of samples stored at the alternating temperatures closely approached those stored at the higher undesirable temperature.

The drip that results from thawing frozen meats often results in a considerable loss in weight. The variation in drip has been attributed to numerous factors. In our sutdies, temperature of freezing was definitely related to the amount of drip. This is due to the fact that in meat frozen at the lower temperatures with resulting fiber breakage, the water was reabsorbed when thawed, whereas at the higher freezing temperatures moisture was withdrawn from the fibers and frozen between them. On thawing, this moisture dripped away as such.

Incomplete studies on nutritive value of pork frozen and stored at 0° F. and 15° F. for 24 weeks, protected with high grade cellophane and exposed, indicated that storage time, temperature, and method of protection had little effect on the growth promoting value of the proteins.

Conclusions

Under the conditions of the experiments reported, the following conclusions can be made.

The freezing and freezer storage of meats result in a product that is practically the same as the fresh meat if all factors involving quality changes are controlled. Fresh meats can be improved in one important factor, that of tenderness. The degree of tenderizing is related to freezing temperature, the lower the temperature of freezing the greater the tenderizing effect. This is due to the extent of fiber splitting and stretching of the connective tissues. Beef aged 5 days and frozen at -10° F. is as tender as similar unfrozen beef samples aged 35 days. Refreezing of beef further increases tenderness. Long freezer storage of beef results in loss of tenderizing effect gained by freezing followed by immediate thawing. Pork and lamb react similarly to beef, with respect to tenderizing due to freezing.

Temperature of storage and fat content of meat influence rate of desiccation in exposed samples. Oxidation of the fat due to enzyme action results in a progressive decrease in desirablity of flavor of fat in meat stored at 18° and 0° F. Oxidative changes vary according to the type of the fat. The "storage life" (period of storage during which the product remains moderately desirable in flavor) of frozen meat samples may be increased by lowering the temperature of storage or by vacuum packing. Vacuum packed meat does not reach the end of its "storage life" in 48 weeks at 18° F. and is similar to samples stored in vacuum at 0° F. Chemical analysis indicates that flavor of lean is directly related to fat content of the lean.

Frequent alternating of freezer storage temperatures between a low desirable temperature and a higher undesirable temperature results in meat that is no better than that held constantly at the latter temperature.

The amount of drip appears to be associated with temperature of freezing, the lower freezing temperatures resulting in less drip.

Freezing and freezer storage of meat results in little loss in growth promoting value of the protein.

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XV

UTILIZATION OF ANIMAL FATS

W. O. Lundberg
Hormel Institute, Austin, Minnesota

Introduction

This paper is concerned primarily with the possibilities for improving the economic status of animal fats through research. Because of its bigness, two treatments of this subject were considered. One alternative was a rather general coverage of the entire subject; the other a detailed consideration of only a few specific research possibilities. For the purposes of this conference it seemed important to achieve a broad and balanced perspective. The first alternative was therefore selected.

It is planned to discuss in a general way the possibilities for increasing the utilization of animal fats (i.e., lard, tallow, and grease)* on the basis of the present economic and technologic status of the fats and oils industry and further, to consider some possibilities for developing entirely new products and new uses. Progress in the first area involves mainly so-called applied or technological research; progress in the second area, in addition, depends to a considerable degree upon new basic scientific researches.

The problem of how to increase the utilization of animal fats commands more than usual interest at present because of the recent declines in prices of animal fats and other oils, and because of the anticipation of an embarrassing surplus production that may continue for some time. There is a tendency to turn to chemists and technologists for a solution to these economic problems through the development of new uses for fats.

Economic Considerations

Closer analysis inevitably leads to the conclusion that the solution to the immediate problems of surpluses and low prices must be found largely in the field of economics rather than in the laboratory.

The production of animal fats is not readily adjusted to demand because animal fats are by-products. Their production is governed to a large degree by the demand for meats. The major oil-bearing crops, on the other hand, are produced largely or mainly for their oils, and their production, to a much greater extent than in animal fats, tends to be geared to demand. Moreover, animal and vegetable fats are to a considerable extent interchangeable as raw materials for some products. Hence, to the extent that they are interchangeable, the over-all production of all fats and oils tends to be geared to total demand, with the adjustment in production occurring most readily in the oil-bearing crops.

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*Except where otherwise indicated, the term "animal fats" as used herein does not include butterfat.

If, therefore, the creation of a new demand for animal fats by the development of some new product is readily offset by substitution of vegetable oils for the animal fats in some other area, and hence offset by an increased production in oil-bearing crops, then, in general, the creation of this new demand will not have a very telling effect upon surpluses and prices in animal fats. Surpluses and prices in animal fats, and in fats and oils generally, will continue to depend mainly upon a combination of such economic factors as postwar agricultural readjustments in Europe, the increased consumption and production of meat in the United States, the growth of soybean production, governmental agricultural policies, and the vagaries of climate which influence the production of both animal and vegetable fats.

It is not intended in the foregoing to discount completely the value of research in improving the economic status of animal fats, but rather to bring into clearer focus those objectives whose attainment through chemical research may be reasonably expected.

Objectives of Research on Animal Fats

One objective of research should be to improve the competitive status of animal fats relative to vegetable oils by increasing the utilization of animal fats in some types of products where vegetable oils are now the principal raw materials.

The manufacture of products which employ vegetable oils as raw materials is the basis for a number of lucrative industries. Because of the interchangeability of animal fats and vegetable oils, it appears that with the solution of a few technical problems, it should be possible to substitute animal fats as raw materials in some of the more highly processed and more profitable types of fat products.

There are, of course, definite limitations, both economic and technological, on the interchangeability of animal and vegetable fats. The most important limitations from the standpoint of the chemist and technologist stem from the differences in chemical composition.

Such limitations are a two-edged sword. Because of them, and because of the by-product status of animal fats, the latter are subject to greater maladjustments between supply and demand, and hence to more precipitous price changes. On the other hand, the skillful research chemist may be able to take advantage of differences in chemical composition to create superior products or devise less costly processing methods with animal fats. Thus, not only can the interchangeability of animal and vegetable fats be employed to advantage, but limitations in such interchangeability may also be exploited.

A second objective of research should be to create for the benefit of mankind entirely new types of products and new uses for products from animal fats. Such research should be aimed at the development of products for which animal fats, by virtue of their composition or because of economic considerations, would be more feasible than vegetable oils or other raw materials.

To be sure, chemists in industries that utilize vegetable oils are working toward analogous objectives. It is perhaps not necessary to point out the extent to which such industries have been successful in their endeavors, and that by the same token much lost ground could be regained by producers of animal fats through research.

113

Relative Importance of Research Objectives

Research aimed at increasing the utilization of animal fats in exist
ing types of products or in substitute products can be designated largel
as short-range research, whereas the creation of new products and of
new uses for products must be regarded as long-range research. In ord
to bring this statement into its true perspective, and in order to evalua
more critically the relative importance of the two objectives that have
been mentioned, it is desirable to examine some statistics concerning
the consumption and production of various fats and oils.

The statistics in Table 1 represent the quantities of fats and oils p
duced and consumed in the United States during 1947. They suggest the
tremendous size of the fat and oil industry, and indicate the huge quanti
ties of animal fats that are involved. Although these figures have change
to some extent in years subsequent to 1947, none of the changes has bee
great enough to alter the conclusions that will be drawn.

The total production of fats and oils from domestic and imported r
materials was more than 10.8 billion pounds, and the total apparent dom
tic consumption was approximately 400 million pounds less. (The differe
ces between production and consumption represent net exports or impor
and changes in year end stocks). Vegetable oils have become an increas

Table I -- Production and Consumption of Fats and Oils, 1947* (in mil-
lions of pounds)

	Production from domestic and imported raw materials	Apparent domestic consumption
Vegetable oils		
Soybean	1540	1448
Cottonseed	1117	1112
Linseed	456	575
Coconut	778	752
All other	540	684
Total	4431	4571
Animal fats (including butter)		
Lard	2432	1995
Inedible tallow and grease	2023	1883
Butter	1637	1634
All other	208	193
Total	6300	5705
Marine oils		
All types	114	168
Total, all categories	10,845	10,444

*Compiled from Industry Report, Fats and Oils, Annual Review, 1947,
U. S. Dept. of Commerce, pg. 27, March, 1948.

ly important part of the industry and constituted about 41% of the total production in 1947. More than 7/8ths of this figure represented the production of four oils. The production of all animal fats, including butter, was about 58% of the total. Lard alone constituted more than 22% of the total production of all fats and oils, and inedible tallow and grease another 19%. Roughly similar percentages are found in the figures for the consumption of the various fats and oils.

Consider now the figures for the production of fat and oil products for 1947, as given in Table 2. The second column gives approximate figures for the animal fats (excluding butterfat) used in producing the quantities of final products given in column 1.

The table reveals that the largest outlets for animal fats were in the products lard and soap. 1.8 billion pounds, or almost 18% of the total production of all fat products, was in the form of lard, as such, without further processing. More than 2.1 billion pounds of fat, mostly inedible,

Table II — Use of Fats and Oils in Products for Civilian Consumption, and Animal Fats used in Manufacture, U. S., 1947* (in millions of pounds)

	Production	Animal fats used (excluding butter fat)
Edible products		
Butter (actual weight)	1606	0
Lard (excluding use in margarine, shortening and nonfood products)	1818	1818
Shortening	1338	166
Oleomargarine (fat content)	580	11
Edible oils (mainly salad and cooking oils, but also including all other edible uses)	997	?
Inedible Products		
Soap	2143	1539
Drying oil products (including paints, varnishes, floor coverings, oilcloth, printing inks, core oils, synthetic resins, insulation, linings, etc.)	973	<1
Other industrial products (including fat equivalent of soaps used in manufactures such as rubber, textiles, etc.)	807	?
Total	10,262	

*Compiled from Agricultural Statistics, 1948, U. S. Dept. of Agriculture, pg. 157-170. The figures in the second column are not strictly applicable to the production figures in the first column, but are good approximations of the true figures.

was used to produce soap, and of this amount about 1.5 billion pounds, or roughly 72% was animal fat.

About 13% of the total production of all fat and oil products, edible and inedible, was in the form of shortening; 6% in oleomargarine; 10% in other edible products; and 10% in drying oil products.

Turning to the last category in the table, it is seen that less than 8% of the total consisted of inedible or so-called industrial products other than soap and drying oil products. By far the biggest portion of this amou represented products in which the fat was present in the form of triglycer ides, fatty acids, or soaps, for example, lubricants and greases, and soap employed in the manufacture of textiles and rubber.

Also included in this category, however, are the numerous other che ical derivatives of fats, including the many distinctly new chemical produc that have been developed in the past 25 years. The production of such new chemical derivatives amounted to much less than 1% of the total productic of fat and oil products. Indeed, their total production is much less than th usual variation in the production of either lard or inedible tallow from on year to another.

After many years of research and development, new chemical deriva tives with new uses have become the basis of a vigorous and fast-growing industry, but as yet can provide only an insignificant outlet for animal fats. Although there is much room for expansion of this outlet, it is evi-dent that an increase in the utilization of animal fats on the basis of the second objective mentioned earlier will be a long range achievement.

In contrast with these long range opportunities are the shorter range opportunities, some of which are reflected in Table 2. One composite ex-ample from the statistics for the edible products will illustrate the point. In the food field, shortening, oleomargarine, and edible oils were prod-uced in a combined amount of 2.9 billion pounds, or about 28% of the total production of fat and oil products. As may be seen in the second column, however, only a very small proportion of these products were derived fro animal fats. Moreover, much of the technological information necessary for a satisfactory and economical conversion of animal fats to these types of products, is already available, and much of the necessary additional information could probably be obtained in a relatively short time.

Hence, relatively short range researches toward the first of the ob-jectives mentioned appear to offer by far the best hope of a more profit-able utilization of animal fats in the near future.

Up to this point, the general objectives of researches on fats and oils and their relative importance have been discussed in the light of general economic considerations to provide an orientation for what follows. The remainder of the discussion will be suggestive of more specific lines of attack for extending the utilization of animal fats in existing types of ed-ible and inedible fat products, and for developing new products, and new uses for existing and new products.

Existing types of edible products.
Referring again to Table 2, 13% of the total production of fat and oil products is in the form of shortening, but only about 1/8th of all shorten-ing has its origin in animal fats. From the technological standpoint, ap-parently very little further research would be required to supply informa-tion which, when combined with the generally available knowledge concern

ing existing processing methods, would make it possible to produce short-
enings from animal fats that are fully the equal of those prepared from ve-
getable oils. New antioxidants and emulsifying agents are now available to
provide high stability and excellent leavening properties.

Oleomargarine constitutes almost 6% of the total fat and oil products.
Here again most of the technological information for the utilization of ani-
mal fats is available and much of the required research consists simply
in improving known processing techniques to obtain a better product or
to make the processes more economical. Table 2 indicates that in 1947
the consumption of butter was approximately three times that of margar-
ine. This, together with the greatly improved competitive position of mar-
garine that will result from legislation of the present Congress, indicates
that margarine could be a very attractive outlet for edible animal fats.

Another 10% of the total products is represented in the category la-
beled edible oils. The proportion of animal fats now used in this category
is not given in the table but is known to be small. The main products in
this classification are salad and cooking oils derived from vegetable
sources.

These, of course, are considerabley more unsaturated than animal
fats. One possibility for the production of more unsaturated oils from ani-
mal fats would depend on the development of a satisfactory and commer-
cially feasible process for the dehydrogenation of fats. Further research
toward this end appears to be worthwhile, not so much to produce more
unsaturated edible oils from animal fats, but to create new drying oils
and drying oil products. A satisfactory dehydrogenation process is not
likely to be achieved easily, however, and this particular accomplishment
will probably await new discoveries in basic organic chemistry.

A second possibility for the development of unsaturated oils from
animal fats lies in the further development of existing processes. In parti-
cular, interesterification accompanied by progressive fractionation during
the interesterification reaction appears to have some potentialities. This
type of process, however, also requires outlets for the more saturated
fractions that are obtained.

No particularly noteworthy development of completely new types of
foods that contain substantial amounts of fat have occurred in recent times.
Such products necessarily involve a combination of the fat with other types
of nutritive ingredients. One product which could utilize not only animal
fats but possibly also animal protein would be a substitute or partial sub-
stitute for milk and cream. Marketing problems might be considerably
greater than the technological problems in developing such a product. One
would predict that with some research it should be possible to create a
product from animal fats and tissue proteins which, though it might not
taste exactly like milk, would look much like milk and could be used for
many of the same purposes. There have been reports that one of the maj-
or processors of vegetable oils is engaged in researches along this line.

Existing types of inedible products.
Soap constituted approximately 21% of all fat and oil products in 1947,
of which about 72% was derived from animal fats, mainly inedible tallow
and grease. The other 28%, about 600 million pounds, was derived from
lauric acid oils, mainly coconut oil. Normally the price of coconut oil
is only slightly above that of inedible tallow and grease. In order to make

117

it possible and profitable to increase the utilization of animal fats in the manufacture of quick-lathering soaps in competition with lauric acid oils, it would be necessary for the chemist to solve several difficult problems. Among them probably would be (1) to find a method of reducing 16- and 18-carbon chains to approximately 12- or 14-carbons, at the same time producing potentially useful by-products, (2) to develop new uses for the by-products, and (3) to obtain a soap that would be markedly superior to those now produced from lauric acid oils. Research in this direction appears to be less promising both technologically and economically than in other fields.

The development of commercial hydrogenation processes has made it possible for vegetable oils to compete elegantly with animal fats, and indeed to virtually supplant animal fats for some uses in the edible field, but the reverse type of process, dehydrogenation, which would make it possible for animal fats to compete with vegetable oils in the production of drying oil products, has not yet been satisfactorily achieved. Hence, although almost 10% of all fat and oil products in 1947 were drying oil products and related substances, virtually none of these came from animal fats. Only a very limited amount of research has been directed toward this end, and the adaptation of animal fats to uses in the drying oil field must be regarded as a long-range project. A potentially useful method of indirect dehydrogneation will be indicated later in this discussion.

New products and new uses
Although the greatest potentialities for the increased utilization of animal fats in the near future lie in the adaptation of animal fats to existing types of edible products or substitutes for such products in competition with other sources of fats and oils, one must not underestimate the great potentialities that lie in the development of entirely new products and new uses. Such potentialities are very important from a long-range point of view.

The chemical industry based on fats and oils is still in its infancy, but is growing steadily. Further, it must be borne in mind that some products that have been and will be developed are worth considerably more than the fats from which they were prepared. Hence, even though the volume of fats employed may be small, the industry in terms of the value of the products may be quite important.

Insofar as the increased utilization of animal fats in chemical derivatives is concerned, the researches must be directed largely toward finding new derivatives and new uses for the derivatives from oleic and palmitic acids. It is necessary to bear in mind constantly not only the competition from vegetable oils, but also from other sources. Such competition exists in the petroleum industry at the present time, and in the future may come also from other possible cheap sources of hydrocarbons, such as lignite and soft coal.

A good illustration of such competition may be found in the synthetic detergent industry, in the early stages of which fats were a relatively important raw material. In 1949 the output of synthetic detergents was between 700 and 800 million pounds, of which all but a very small fraction was derived from pertroleum rather than triglyceride fats and oils. Even in the development of edible products, the possible competition from petroleum and other hydrocarbon sources cannot be indefinitely

overlooked. The production of large quantities of synthetic fats for edible purposes from brown coal by the Germans is too well known to require more than passing mention.

Many new types of chemical derivatives from fats are possible, and many of the derivatives already developed have been found to be extremely versatile. It is obviously impossible to discuss intelligently the uses for derivatives that have not yet been synthesized, since such uses can be devised only after the chemical and physical properties of the derivatives have become known. The potentialities for uses of chemical derivatives are suggested by the applications of some of the derivatives of fats and oils that have been developed commercially in recent years. Included are uses as or in: detergents and wetting agents, drying oils, plastics and resins, emulsifiers, flotation agents, hard and soft waxes, plasticizers, penetration agents for leather, tack reducing agents, textile softeners, antifoaming agents, metallic soaps, rain-proofing for textiles, rubber substitutes, insecticides and insect repellants, fungicides and germicides, metal working and finishing compounds, special lubricants, high boiling solvents, essences, perfumes, flavors, cosmetics, salves, creams, dyestuffs, pharmaceuticals, and many others.

It is also impossible to delve adequately into the many derivatives that may be considered for commercial development. By considering the chemical reactions of fats, upon which the development of new derivatives is necessarily based, it will be possible to point to some chemical properties that have already been utilized in new derivatives, and to others that yet remain to be utilized commercially. Because most of the new and projected derivatives and uses for fats are related primarily to the chemistry of the fatty acid portion of fats and oils, and since the fatty acid portion constitutes approximately 95% of the triglyceride molecule, derivatives of the glycerol portion of fats and oils will not be considered.

The chemist who is seeking to develop commercially useful derivatives must consider those chemical aspects in which animal fats are unique in relation to other possible competitive materials such as petroleum. In so doing, he is faced with three facts:

1. Fatty acids are essentially hydrocarbon materials possessing straight chain molecules of relatively uniform length, 16 and 18 carbons.

2. In addition to the hydrocarbon moiety, they contain a reactive carboxyl group which is always located at an end of the molecule.

3. The oleic acid of animal fats contains another reactive center in the form of a double linkage in the 9, 10 carbon position which occurs only in the cis geometric configuration. Palmitic and stearic acids are completely saturated and are therefore relatively inert in all portions of the molecule except the carboxyl group.

Even though fatty acids from animal fats are far less complex mixtures than petroleum, the significance of the first of these facts should not be overestimated. The petroleum industry has overcome many obstacles by its advanced development of fractionation processes. Even though various well known commercial methods for the fractionation of fatty acids have been developed in recent years, the example of the petroleum industry should be followed in carrying on further researches to develop fractionation processes. An increasing availability of purified fatty acids of fatty acid concentrates will lead to more extensive uses for the fatty acids themselves and, further, to a greater develop-

ment of fatty acid derivatives.

Derivatives based on reactions of the carboxyl group

Many of the common reactions of the carboxyl group have been adap
to commercial practice and are employed in the synthesis of various der
vatives. The production of metallic soaps for use primarily in lubricants
and paint driers is well known.

Various types of esters are becoming increasingly important. Some
simple esters are used as plasticizers, others as high boiling solvents.
Pentaerythritol esters of fatty acids and oil modified alkyd resins are
examples of esters that have achieved prominence in the drying oil field.
Esters of polyhydric alchohols such as sorbitol are finding application as
emulsifiers and also other uses in foods and industrial products. It has
been suggested that fatty acid esters of unsaturated alcohols may be used
as starting materials for new types of polymers.

Acyl halides have proved to be useful chemical intermediates for the
production of some types of esters. Thus they have been used to produce
fatty acid derivatives of cellulose for use in synthetic fibers. Other use-
ful esters of fatty acids from relatively inert hydroxylated substances can
probably be prepared by using the acyl halides.

The pioneering work of Armour and Company in developing new pro-
ducts through reactions of the carboxyl group are well known to all. The co
mercial development of such derivatives as amides, nitriles, amines, am
acetates, and quaternary amines, and the development of many uses for th
products is a fascinating illustration of the achievements that are possibl
in this field. The application of the amines and other derivatives as ore fl
tation agents in the concentration of low grade iron ores could at some
future date achieve very considerable proportions.

The fatty alcohols produced by hydrogenolysis are another relatively
important class of derivatives. Only a small proportion of detergents and
surface active agents are now produced from fatty alcohols derived from
tri-glyceride fats and oils, but with more pure individual fatty acids from
tallow and other sources becoming available, the possibility of expanding
the utilization of fatty acids in detergent production for special uses shoul
be further explored.

Other possible derivatives of the carboxyl group are aldehydes. Here
is a case where a large potential use in the resin industry already is evi-
dent, and what is needed is the development of a practical commercial
method for the production of aldehydes from fatty acids.

Ketones and hydrocarbons derived from fatty acids through reactions
of the carboxyl group are other products for which research may find
suitable uses and methods of production.

Derivatives produced by reactions involving unsaturated linkages.

The reactivity of the unsaturated centers in fatty acids has not yet
been as fully exploited as the reactivity of the carboxyl group in the crea-
tion of new derivatives, but in recent years a considerable amount of pro-
gress has been made.

Halogens and other inorganic substances can be added at the double
bonds. Up to the present time halogen derivatives have found a limited
application in the lubricating field, but their use as intermediates has
been little explored and many possibilities exist. Sulfated and sulfonated

derivatives of fatty acid derivatives are already well known and used in textile, leather, and cosmetic industries, and other inorganic reagents can undoubtedly be added to the double bonds to produce new and useful derivatives.

Of course, the double bonds of fatty acids are susceptible to oxidation, and under proper conditions a preponderance of dihydroxy derivatives or keto-hydroxy compounds may be obtained. Because of the reactivity of the hydroxyl groups, such compounds are potentially important intermediates and many useful derivatives would be possible. However, cheaper commercial processes for the production of relatively pure hydroxy derivatives are necessary.

More drastic oxidation results in cission of the double bond and produces aldehydes or acids. The dibasic acids in particular are potentially valuable in the production of polymers. Azelaic acid is already produced commercially from oleic acid. A great increase in its use simply awaits the development of a cheaper process for its production.

In recent years it has been found that not only may reactions occur at the double bond but the carbons in the alpha position relative to the double bonds are also highly reactive in some instances. Thus in autooxidation, the addition of oxygen occurs primarily at the alpha carbon.

Ordinarily the autoxidation of fats is considered to be of interest only in relation to the problems of rancidity or to the drying of oils. However, a number of fundamental studies of the autoxidation of fatty acids have been carried on recently, including some in the laboratories of the American Meat Institute Foundation and others at the Hormel Institute. In some of these studies, derivatives obtained by autoxidation have been concentrated and studied. It has been found that under properly controlled conditions, it is possible to obtain very reactive peroxides of individual fatty acids in highly concentrated form. Concentrates of oleate peroxides have been found to be extremely effective polymerization catalysts for some polymerization reactions. Such uses of the fat peroxides would of course utilize only very limited amounts of fat.

However, peroxides may also be reduced easily to hydroxy derivatives, and since these hydroxy derivatives still possess their original content of double bonds, they also are reactive substances and can serve as intermediates for many types of products. Further, the hydroxy derivatives may be dehydrated, yielding new double bonds. Moreover, in this sequence of reactions a high proportion of conjugated double bond systems will develop; thus oleic acid can yield a high proportion of conjugated dienoic acids which, of course, like the dehydrated acids of castor oil, are highly reactive.

The double bonds of fatty acids, and the alpha positions relative to the double bonds, are reactive toward various organic substances also. Such addition reactions have already assumed considerable importance in the production of polymers. One example is the addition of maleic anhydride, a reaction which may occur in several ways. Almost limitless possibilities exist for the synthesis of other addition compounds that may be useful in polymer production.

Another interesting addition reaction involves the combination of two fatty acids to form a dimer acid. Dimer acids have recently been found to be useful substitutes for palm oil in tin plating and are also useful in some greases and alkyd resins. Since dimer acids are dibasic they can be

121

used in the production of polymers. Among such polymers are the polya-
mide resins which are now used as sealing compounds for packaging.

All of these reactions, insofar as they can be particularly adapted to
animal fats and fatty acids, can improve the economic status of animal
fats.

Other reactions

Although reactions involving either the carboxyl group or the unsatur-
ated centers appear to be by far the most important in the development of
new derivatives, one cannot altogether overlook the possibilities of other
reactions. Useful derivatives may be possible by substitution reactions on
the alpha carbon relative to the carboxyl group. And, although pyrolysis of
fats under ordinary circumstances yields a complex mixture of products,
it is possible that catalytic processes could be found which would give rel-
atively high yields of a few valuable products by thermal decomposition.
Catalytic processes may of course be important to the development of
most of the derivatives that have been considered.

Minor constituents.

Animal fats contain small amounts of various constituents other than
triglycerides. The potentialities of these minor constituents have never
been adequately investigated. They may for example contain unknown
nutritional factors which if discovered and identified would stimulate the
utilization of animal fats in edible products. They may also contain base
materials for the development of therapeutic agents and other pharma-
ceuticals. As a starting point, a thorough investigation of the composition
of the minor constituents of animal fats appears to be needed.

Summary and conclusion

It has been indicated that the greatest improvement in the economic
status of animal fats in the near future can be achieved through their utili-
zation in types of products that already exist, particularly in the edible
field. The demand for animal fats may also be greatly increased in the
more distant future through the development of new products with new uses,
especially new chemicals for industrial utilization. The latter possibili-
ties, however, are largely contingent upon new basic researches in the
chemistry of fats and oils.

President DuBridge of the California Institute of Technology recently
warned that of the countless millions of dollars spent each year to advance
technology in the United States, dangerously little was going into basic
scientific research. He also pointed out that "the aim of science is to dis-
cover new knowledge and new principles. The aim of technology is to in-
vent new devices, new machines, new processes, new techniques, and in
the long run the new developments in technology will be based only on the
new knowledge uncovered by science."

It is therefore to be hoped that in efforts to increase the utilization of
animal fats, the importance of basic scientific research will not be over-
looked.

XVI

FOOD STABILITY RESEARCH AT THE
QUARTERMASTER FOOD & CONTAINER INSTITUTE

H. L. Fevold
Quartermaster Food & Container Institute
For the Armed Forces

The function of the QMF&CI, as most of you know, is to develop food items which have the best possible characteristics for use in Armed Forces rations. The three most important characteristics to be considered are utility, stability, and acceptability. In order to fulfill that function, a very extensive program has been underway for some time, and we have made some small beginnings in getting a food research program underway within the Institute. It is the latter program with which we will deal in this discussion. The external research program which was represented by two or three papers on your program last year will not be dealt with except incidentally.

At least two lines of work are of major interest in our research program. One of these derives from the fact that items which make up rations for the Armed Forces must have stability characteristics which are more difficult to obtain than those which are necessary for the civilian market. Many items which for the civilian trade must be stable for relatively short periods of time at comparatively low temperatures or even under refrigeration, should, in order to possess the optimum characteristics, be stable for relatively long periods of time at elevated temperatures — some for 6 months, or more at 100° F. Consequently, many reactions which can be fairly well controlled under the conditions of normal commercial practice become increasingly important factors in the problem of maintaining acceptability or edibility of Armed Forces rations and ration items. Study of deterioration, the fundamental mechanisms of deterioration, and development of methods for decreasing the rate of, or optimally of blocking the reactions completely, are, therefore, of paramount importance.

In conducting studies on food quality and food deterioration, we are fortunate at the Quartermaster Food and Container Institute to have a combination of skills with which to evaluate deteriorative changes not only from the chemical and physical, but from the sensory and organoleptic standpoint as well. We have what we believe to be an excellent food acceptance laboratory, and new and perfected methods for such evaluation results from the efforts of our food acceptance group. In conducting chemical and sensory evaluations of a ration item simultaneously, a correlation of the two types of data becomes possible, and a fuller understanding of the factors responsible for the loss of quality results. This leads up to, or is part of, the second line of work which is of practical importance to the Quartermaster Corps.

At the present time, there are very few physical or chemical tests

which can be used as indicators of quality to the consumer. Sensory evaluations can be used, but in many cases are not feasible and in all cases they are time-consuming and expensive. If adequate correlation could be made between chemical changes and loss of quality as determined by sensory methods, chemical quality indices might be established which could be used in our specifications for quality control purposes by the inspection agency. At the QMF&CI, we are in a position to establish such standards where possible, and where they are lacking.

It has been necessary, due to the limited personnel which we have had available for research purposes, to limit our activities to those problems and commodities which have seemed to our technical and military staffs to have the highest priority as regards needs for the development of satisfactory end items or rations. Consequently, the major activities of our stability research, for example, have been directed toward a solution of some problems connected with the improvement of dehydrated milk for beverage purposes. However, I feel that a good deal of the work going on is of interest in, and has applicability to, problems related to stability of other types of items, such as meat as well. I am, therefore, reviewing briefly for you some of the work phases in which we are currently engaged.

We do have some work which deals directly with meat. This is a continuation of the work initiated by Professor Seelock at Iowa State under contract with the QMC and which was presented by him to this group a year ago. His report dealt with the breakdown of proteins during aging of chicken muscle, as indicated by a marked increase in non-protein nitrogen. The possibility of a relationship to the tenderizing process was discussed. Lieutenant Yare, who worked with Professor Seelock on this problem, has been in our laboratory since last July, and has extended that work to include beef muscle. His work is far from completed, but in general he finds the same qualitative picture in beef as in chicken muscle. However, beef muscle apparently contains more enzymes, or at least indicates greater enzymic activity, than does chicken meat, since non-protein nitrogen increases at a greater rate during curing of beef. Experimental work is planned to determine what proteins are concerned in this change. Mr. Yare is also extending his observations to determining the changes in non-protein nitrogen during various processing procedures and relating the changes to the structural and organic properties of the product. The results seem likely to be quite instructive.

Some aspects of lipid deterioration are being studied, particularly as related to deterioration of milk fat. Until the past year, work was conducted under contract to the QMC at the Meat Institute and University of Minnesota on the fundamental aspects of lipid deterioration. This work was carried out on model systems using spectrophotometric methods, and very interesting information, both with regard to mechanisms of oxidation and some leads as to probable methods of inhibiting those changes, resulted from that work. We have initiated work at the Institute using infrared spectroscopy in an attempt to use this technique in learning more about the deteriorative changes which take place in milk fat during processing and storage of dried milk. Mr. Henick, a former employee of the Meat Institute, is carrying on that work, and has obtained results which give worthwhile information regarding the loss of quality. It seems that further effort along that line will be worth while both as applied to milk fat and probably

to other fats as well.

Briefly, the information as we have it today is this: Infrared and organoleptic characterization of stored whole milk fat showed that there were no observable changes in the infrared spectrum, even after definite quality changes were obvious, as determined by sensory methods. However, if the deteriorated milk fat was steam-distilled, changes in infrared absorption of the distillate were apparent before organoleptic changes were detectable. That the alteration of the absorption characteristics was related to deteriorative changes was indicated by the fact that these same changes were magnified on further storage when the sample had deteriorated organoleptically.

Principal changes in absorption occur near 3 microns where bonded -OH groups absorb and between 5.5 and 6.0 microns where C = O groups absorb. The absorption at 3 microns became broader and shifted toward longer wave length as storage time of the sample increased. This band was attributed to a state of association in -OOH groups in hydroperoxides found during autoxidation.

The absorption between 5.5 and 6.0 microns shows considerable change as the sample oxidizes. In the fresh fat it was resolvable into only two peaks — one at 5.80 microns due probably to diacetyl, and one at 5.75 microns, the cause of which is not yet known. On storage the band at 5.80 microns remains quite constant, that at 5.75 either disappears or shifts to 5.70, and two new bands appear at 5.84 and at 5.91. The new prominent peak at 5.91 microns, together with a weaker band at 6.10 microns, is taken as evidence of a conjugated enone or enol which may be responsible for much of the off-flavor in oxidized milk fat.

It is much too early to draw any conclusions from the work. However, it appears that under these conditions the organoleptic deteriorative changes are detected very early by the infra absorption characteristics and may prove to be an objective index to detect quality deterioration.

Another work phase specifically related to milk fat, but which may be of interest in relation to animal body fat, is one being carried on by Lt. Handwerk. This work was initiated at Iowa State College under the direction of Professor Bird as contractual research with the QMC. The work was originally planned as a study of the tocopherol content of milk, particularly as related to diet and to deterioration. However, difficulty was encountered with the methods which are available for determining tocopherols, and the study has resolved itself into the development of adequate methodology. Briefly, the difficulty is due to the development of oxidation products during storage which interfere with the determination.

As an example of work (which we hope to expand in the future) designed to develop chemical criteria which may be used to evaluate the quality of the product, a work phase presently underway might be cited. This work phase deals again with dehydrated milk, and its specific aim is to correlate chemical and physical chemical properties with organoleptic quality with the hope of selecting those objective changes as quality indicators which correlate well with the organoleptic quality. None of the requirements presently set forth in the specification for dehydrated milk, such as bacterial count, butter fat content, moisture, acidity, solubility, or copper and iron content appear to be adequate. The work is centered around certain chemical changes which have been shown to take place during dehydration and storage, and which are believed to be responsible for the develop-
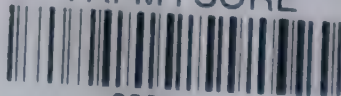
ment of specific off-flavors and other specific undesirable characteristics of dehydrated milk. This information has resulted to a large extent from work supported by OQMG at the Dairy Departments of Universities of Minnesota, Ohio State, Illinois, and Pennsylvania State, and involves changes indicated by total protein solubility, soluble whey nitrogen, fluorescence, reducing capacity, and phosphatase activity.

This work is far from completed, and no final correlations and conclusions have been made. However, there appear to be some interesting relationships among soluble whey protein nitrogen, heat treatment, and acceptability data. Initial quality is highest when the heat treatment is moderate and the amount of soluble whey protein is intermediate between the high and low values. This is due to the fact that high heat treatment results in the liberation of free -SH groups and the so-called cooked flavor. However, on storage of high heat-treated powders, an increase in soluble protein takes place, the cause of which has not been determined, but may be due to reversal of denaturation or to hydrolysis. Also, the high heat-treated milks retain their initial quality better during storage, almost certainly due to the change in oxidation-reduction potential and the reducing properties of the -SH groups.

Microbiological work is in progress consisting of a study of lipolytic microorganisms and their role in food deterioration. Published work has indicated that bacterial metabolic products may result in food deterioration even after the viable bacterial count is reduced essentially to zero. It is believed that a study of lipoclastic microorganisms, their enzyme systems, and the metabolic end products produced by their action on lipids will provide valuable information in helping to improve the stability of dairy and meat products and other foodstuffs which contain various fats and oils.

To date, the work has consisted of developing methods for measuring lipolytic activity which have the necessary accuracy and precision. A stable, finely dispersed nutrient oil emulsion has been developed which maintains an effective concentration of the lipid for efficient utilization by the microorganism. Soybean phospholipids have been found to be particularly useful as a stabilizer, since they maintain the lipid in a stable state during and following sterilization by autoclaving, have no antibacterial action and are not acted on by the lypolytic bacteria used in this study. Further, the optimal conditions for lypolytic action have been studied. Protein, lipid, and phosphate concentrations are important factors for lypolytic activity. Information presently available does not prove, though it points strongly to enzymic lypolytic activity after the death of the microorganism.

Work is continuing on trace metal catalysis. Many of you are acquainted I am sure, with the work which John Thompson initiated at the Laboratory on the role of copper in lipid deterioration. It was established that copper combined with protein in the presence of ascorbic acid to form a complex which was much more active catalytically than was ionic copper. The possibility of a relationship of the copper protein complex to copper-containing enzymes was pointed out. Mr. Coleman is expanding that work to include various proteins and amino acids. In so doing, he has found that some of the amino acids inhibit the catalytic effect of copper. Other copper inactivators are being sought. Conalbumin, one of the egg white proteins which has the peculiar characteristic of binding iron very firmly, also binds copper and is an effective inhibitor of copper catalysis in a model system. A quan-
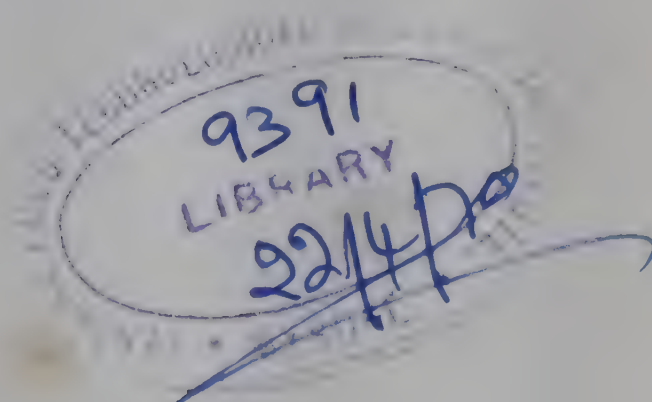
titative method for measuring the catalytic activity of copper has been developed which is very useful in this work. The method is based on oxidation of ascorbic acid.

As I have already indicated, food acceptance work is being actively and effectively prosecuted under the supervision of Mr. Peryam. New sensory methods are being developed and applied to the varied problems which arise, both in research and development work. Some of the problems being worked on at the present time are: (1) quality evaluation of natural and synthetic peppers; (2) effect of pepper on acceptability of ration items; (3) effect of sodium glutamate on acceptability of ration items; (4) relationship of taste and odor sensitivity to appetite and/or satiety; and (5) survey of Field Ration A master menus to determine relatively unacceptable food items in order to eliminate them from the menus, or reducing the procurement of such items.

Nutrition work at the Institute under Dr. Spector's supervision is concerned mainly with the problems of assuring the adequacy of Armed Forces rations in all known essential nutrients. To that end, biological evaluations of rations are being conducted in a manner similar to the method used by Elvehjem and which was reported to you at your meeting last year. Microbiological methods are being used and new microbiological methods developed for evaluation of the nutritional adequacy of proteins. Possible toxicological effects of certain deterioration products are also being investigated.

Throughout this brief discussion, I have pointed out from time to time the rather close relationship of our internal research program to the contractual work sponsored by the OQMG at various universities and research institutes. In many instances, the work carried out externally has indicated the direction our work at the Institute should take, and we hope that in the future we shall be adequately staffed so as to be able to take better advantage of the information furnished by our outside collaborators in application to the solution of our problems.